
# Life, Death, and Cell Proliferation

## A product guide to Apoptosis, Cell Cycle and Cytotoxicity

**BD Biosciences**
Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen

BD

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*Reactivity:*
*Bab:baboon; Bov:Bovine; C: Chicken; Cyno: Cynomolgus; D: Dog; Dros: Drosophila; F: Frog; GPig: Guinea Pig; Gt: Goat; Ham: Hamster; Hs: Horse; Hu: Human; Koa: Koala; Mam: Mammalian; Ms: Mouse; Pg: Pigeon; Qua: Quail; R: Rat; Rb: Rabbit; Rhe: Rhesus; Tr: Trout; Xen: Xenopus*

*Applications:*
*Block: Blocking; FA: Functional Assay; FCM: Flow Cytometry; IC/FCM: Intracellular Flow Cytometry; IF: Immunofluorescence; IHC: Immunohistochemistry; IP: Immunoprecipitation; Neu: Neutralizing; RPA: Ribonuclease Protection Assay; WB: Western Blot*

*For Research Use Only. Not for use in diagnostic or therapeutic procedures.*
*All applications are either tested in-house or reported in the literature. See Technical Data Sheets for details.*

*BD flow cytometers are class I (1) laser products.*
The Alexa Fluor® dye and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc., for research use only or as analyte specific reagents, except for use in combination with microarrays and high content screening, and are covered by pending and issued patents.

# Apoptosis

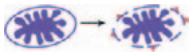
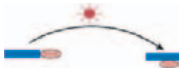
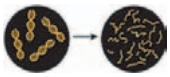
## Introduction

Programmed cell death or Apoptosis is a normal physiological process that occurs during embryonic development and in the maintenance of tissue homeostasis. The term *apoptosis*, from the Greek word for "falling off" of leaves from a tree, is used to describe a process in which a cell actively participates in its own destructive processes. The apoptotic program is characterized by certain morphological features. These include changes in the plasma membrane such as loss of membrane symmetry and loss of membrane attachment, a condensation of the cytoplasm and nucleus, and internucleosomal cleavage of DNA. In the final stages, the dying cells become fragmented into "apoptotic bodies", which are rapidly eliminated by phagocytic cells without eliciting significant inflammatory damage to surrounding cells. Inappropriate induction of apoptosis has widespread pathological implications and has been associated with diseases such as neurodegenerative diseases, graft-versus-host diseases, transplant rejection, cancer, and AIDS.

The study of apoptosis has never been more important than it is today. Along with this intensive study has come the development of a multitude of methods to detect apoptotic cells and dissect the mechanisms of apoptosis. It is important to note that not every cell type will display all the classical features of apoptosis. As such, studies designed to measure multiple aspects of apoptosis may be required to analyze and confirm the mechanism of cell death.

BD Biosciences offers a full line of apoptosis detection tools and technologies for measuring indicators of apoptosis at different stages. These reagents and technologies are based on the following distinctive features of apoptosis:

- Plasma Membrane Alterations
- Mitochondrial Changes
- Activation of Caspases
- DNA Fragmentation

| Feature Measured | Assays | Key Features |
|---|---|---|
| Phosphatidylserine Exposure  | **Annexin V binding assay**<br>• Single conjugates<br>• Annexin V Kits | • Detects early Apoptosis markers<br>• Quick and easy<br>• Flow cytometry or Immunofluorecsence application |
| Mitochondrial Changes  | • BD Mitoscreen Kit | • Fast, easy, single cell resolution by flow cytometry or fluorescent microscopy |
| Caspase Activation  | • Caspase Activity Assay Kits and Reagents | • Quick and easy, uses spectrofluorometry |
| | • Active Caspase -3 immunoassays (BD CBA, ELISA) | • Specific, quantitative, flow cytometry and ELISA application |
| DNA - Fragmentation  | • APO-BrdU™ TUNEL ASSAY<br>• APO-DIRECT™ TUNEL ASSAY | • Works adherent cells, single cell resolution in conjuunction with cell cycle analysis by flow cytometry |

With an overwhelming number of available techniques and products, selecting the most appropriate method is often difficult. To help make this choice easier, the overview above summarizes commercially available assays from a biological perspective. More details on each assay are included in the separate product overviews. A decision tree on page 30, shows a breakdown according to sample type and the method used, and is intended to help researchers determine the most suitable method for their particular experiment.

# Apoptosis

## Plasma Membrane Alterations

### Annexin V Reagents and Kits

- Detect one of the earliest events of apoptosis in living cells
- Perform this simple assay in just 10 minutes
- Use flow cytometry and fluorescence microscopy

Changes in the plasma membrane are one of the earliest features of apoptosis. In apoptotic cells, the membrane phospholipid, phosphatidylserine (PS), is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. Annexin V is a 35 – 36 kD $Ca^{2+}$-dependent phospholipid-binding protein that has a high affinity for PS and binds to cells with exposed PS. Annexin V may be conjugated to fluorochromes such as FITC and PE, or to biotin or tagged with EGFP (enhanced green fluorescent protein). These formats retain their high affinity for PS and thus serve as sensitive probes for flow cytometric analysis of cells undergoing apoptosis.

Because externalization of PS occurs in the earlier stages of apoptosis, Annexin V staining can identify apoptosis at an earlier stage than assays based on nuclear changes such as DNA fragmentation. Annexin V staining precedes the loss of membrane integrity which accompanies the latest stages of cell death resulting from either apoptotic or necrotic processes. Therefore, staining with Annexin V in conjunction with vital dyes such as propidium iodide (PI) or 7-amino-actinomycin D (7-AAD) allows the researcher to identify early apoptotic cells (Annexin V positive, PI negative) from those that are in the later stages of apoptosis or already dead (Annexin V positive, PI positive).

The assay does not distinguish between cells that have undergone an apoptotic death from those that have died as a result of a necrotic pathway, because both dead cell types will stain positively with both Annexin V and PI. Performing a time-course of Annexin staining allows one to track cells as they undergo apoptosis.

BD Biosciences offers Annexin V reagents, individually or conveniently packaged in our Annexin V Apoptosis Detection Kits:

The BD Pharmingen™ Annexin V Detection Kits are flow cytometry based kits for quantitative analysis of apoptotic cells and contain all the critical reagents required for the staining procedure.

In addition, a wide selection of fluorochrome conjugates of Annexin V are available as individual reagents, as are the viability dyes PI and 7-AAD, thus allowing flexibility for experimental design using different a fluorescence channel for flow cytometry.

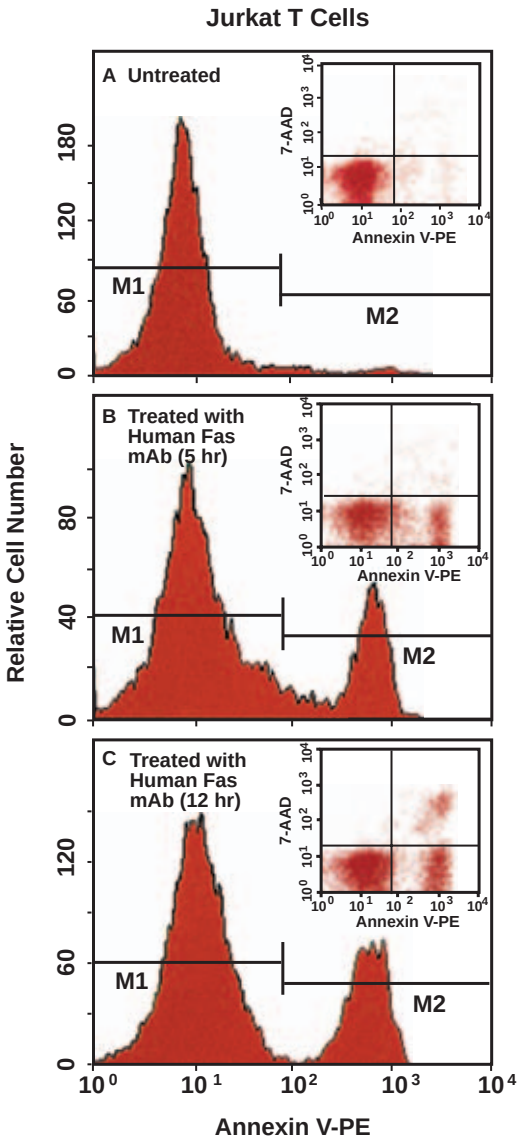



*Figure 1. Flow Cytometric Analysis of Apoptotic Cells Using Annexin V–PE. Jurkat T cells were left untreated (A) or treated for 5 hr (B) or 12 hr (C) with anti-human Fas mAb (Clone DX2, Cat. No. 555670) and Protein G. Cells were incubated with Annexin V–PE in a buffer containing 7–AAD (Cat. No. 555816) and analyzed by flow cytometry. Untreated cells were primarily Annexin V–PE and 7–AAD negative, indicating that they were viable and not undergoing apoptosis. After a 5 hr treatment with DX2, there were two populations of cells: Cells undergoing apoptosis (Annexin V–PE positive, 7–AAD negative), and cells that were viable and not undergoing apoptosis (Annexin V–PE and 7–AAD negative). After a 12 hr treatment with DX2, three populations of cells were identified: Cells that had already died or were in a late stage of apoptosis (Annexin V–PE and 7–AAD positive), cells undergoing apoptosis (Annexin V-PE positive and 7–AAD negative), and cells that were viable and not undergoing apoptosis (Annexin V–PE, 7–AAD negative).*

The Annexin V FITC Fluorescence Microscopy Kit (Cat. No. 550911) is recommended for detection of apoptosis in adherent cells. The fast and simple staining protocol provides a visual confirmation of cells in the early stages of apoptosis shortly after the induction of cell death. Detailed protocols are available in kit-and individual reagent technical data sheets. A general protocol is available online: **bdbiosciences.com/pharmingen/protocols/Annexin_V_Staining.shtml**

## Annexin V Reagents

| DESCRIPTION | FORMAT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|
| **Annexin V** | APC | FCM | 100 tests | **550474** |
| | | | 200 tests | **550475** |
| **Annexin V** | Biotin | FCM | 100 tests | **556418** |
| | | | 200 tests | **556417** |
| **Annexin V** | Cy5 | FCM | 100 tests | **559933** |
| | | | 200 tests | **559934** |
| **Annexin V** | Cy5.5 | FCM | 100 tests | **559935** |
| **Annexin V** | FITC | FCM | 100 tests | **556420** |
| | | | 200 tests | **556419** |
| **Annexin V** | PE | FCM | 100 tests | **556422** |
| | | | 200 tests | **556421** |
| **Annexin V** | Purified | Block, FCM | 100 µg | **556416** |
| **Annexin V Binding Buffer, 10X Concentrate** | | FM, FCM | 50 ml | **556454** |

## Annexin V Kits for Flow Cytometry

| DESCRIPTION | FORMAT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|
| **Annexin V FITC Apoptosis Detection Kit I** | Kit | FCM | 100 tests | **556547** |
| **Annexin V FITC Apoptosis Detection Kit II** | Kit | FCM | 100 tests | **556570** |
| **Annexin V PE Apoptosis Detection Kit I** | Kit | FCM | 100 tests | **559763** |

## Annexin V Kits for Fluorescence Microscopy

| DESCRIPTION | FORMAT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|
| **Annexin V FITC Fluorescence Microscopy Kit** | Kit | FM | 25 Slides | **550911** |

## Mitochondrial Changes

### Mitochondrial Membrane Potential - $\Delta\psi_m$

- Detect changes in mitochondrial membrane potential
- Suitable for flow cytometry or fluorescence microscopy
- Easy to use and interpret

An abundance of data suggests that mitochondria play a critical role in apoptosis by releasing cytochrome c and other proteins essential for activation of pro-caspase-9 and execution of apoptosis. As such, assays designed to monitor the functional status of mitochondria are emerging as useful tools for elucidating the role of mitochondria in apoptosis, cell cycle, and other cellular processes.

One of the earliest indicators of mitochondrial mediated apoptosis is a change in the mitochondrial membrane potential.[1] This depolarization is followed by an increase in mitochondrial membrane permeability and the subsequent release of various soluble intermembrane proteins into the cytoplasm, such as cytochrome c, AIF, Smac/DIABLO, and various pro-caspases. The BD™ MitoScreen Kit (containing JC-1) provides fluorescence-based assay to detect and measure apoptotic cells during the early stages of apoptosis.

The membrane-permeable lipophilic cationic dye, JC-1 used as a probe of $\Delta\psi_m$ .[2-4] This dye can exist in two different states: aggregates or monomers, and each state is characterized with a different emission spectra. Healthy, normal mitochondria take up dye, leading to formation of aggregates resulting in high red fluorescence measured in FL2. The formation of JC-1 aggregates are reversible. Transition of mitochondria from polarized to depolarized (due to apoptosis or other physiological events) results in leaking of dye as monomers out of the mitochondria into the cytoplasm, resulting in a decrease of red fluorescence.

JC-1 do not accumulate in mitochondria with depolarized $\Delta\psi_m$ and remain in the cytoplasm as monomers resulting in low red fluorescence.

The BD™ Mitoscreen Kit provides a simple, quick method for obtaining quantitative data concerning mitochondrial membrane potential.

| DESCRIPTION | FORMAT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|
| **BD MitoScreen (JC-1)** | Kit | FCM | 100 tests | **551302** |


**References**

1. Green DR, Reed JC.1998. Mitochondria and apoptosis. *Science*. 281 (5381):1309-12
2. LeMasters, J.J., A.L. Nieminen, T. Qian, L.C. Trost, S.P. Elmore, Y. Nishimure, R.A. Crowe, W.E.. Cascio, D.A. Brenner, and B. Herman. 1998. The mitochondrial permeability transition in cell death: A common mechanism in necrosis, apoptosis, and autophagy. *Biochim. Biophys. Acta*. 1366:177-196.
3. Reers, M., S.T. Smiley, C. Mottola-Hartshorn, A. Chen, M. Lin, and L.B. Chen. 1995. Mitochondrial membrane potential monitored by JC-1 dye. *Methods Enzymol*. 260:406-417.
4. Cossarizza, A., M. Baccarani-Contri, G. Kalashnikova, and C. Franceschi. 1993. A new method for the cytofluorimetric analysis of mitochondrial membrane potential using the J-aggregate forming lipophilic cationic 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide (JC-1). *Biochem. Biophs. Res. Comm*. 197:40-45.

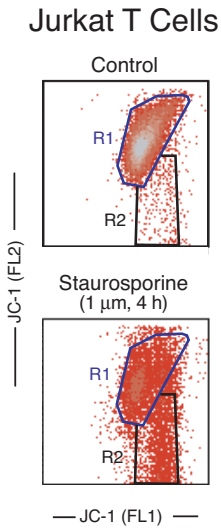




***Figure 2.*** *Jurkat T cells were either left untreated (control) or treated with staurosporine to induce apoptosis. Cells were stained with *BD™ MitoScreen (JC-1) and analyzed on a BD FACSCalibur™ (BDIS, San Jose, CA)*

**Depolarized $\Delta\psi_m$ Corresponds Primarily to CD4⁺/CD8⁺ Thymocytes**

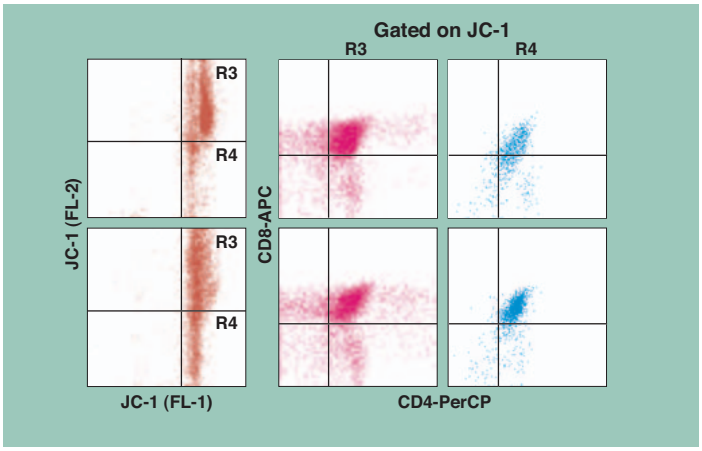



***Figure 3.*** *Mouse thymocytes were isolated from the thymus of a 6 week old BALB/c mouse and left untreated or treated with a mouse Fas antibody [clone Jo2, Cat. No. 554254; 2 µg/ml (1 x $10^6$ cells/ml)] + Protein G [2 µg/ml (1 x $10^6$ cells/ml)] for 3 hr to induce apoptosis. Cells were stained with JC-1 (MitoScreen, Cat. No. 551302), washed, and then stained with CD4-PerCP (Clone RM4-5, Cat. No. 553052) and CD8-APC (Clone 53-6.7, Cat. No. 553035). Cells were analyzed on a BD FACSCalibur™ flow cytometer.*

## Activation of Caspases

One of the earliest and most consistently observed features of apoptosis is the activation of a series of cytosolic proteases, the caspases, which cleave multiple protein substrates *en masse*, leading to the loss of cellular structure and function, and ultimately resulting in cell death.[1] In particular, caspases -3, -8 and -9 have been implicated in apoptosis: caspase-9 in the mitochondrial pathway, caspase-8 in the Fas/CD95 pathway, and caspase-3 more downstream, being activated by multiple pathways.

### Active Caspase-3 and Other Caspases

- Western blot
- Immunohistochemistry
- Immunoprecipitation
- Flow cytometry

Active caspase-3 has emerged as a powerful marker of cells undergoing apoptosis, and its presence is considered to be indicative of apoptosis.[2, 3] caspase-3 is a key protease that becomes activated during the early stages of apoptosis. In its active form, it proteolytically cleaves and activates other caspases, as well as relevant targets in the cytoplasm (eg, D4-GDI) and nucleus (eg, PARP). Numerous antibodies from BD Biosciences recognize the active form of caspase-3 and are useful tools for assessing its activation status using techniques ranging from ELISA, immunohistochemistry, and immunoprecipitation to flow cytometry and BD™ CBA's (cytometric bead array). These techniques allow multiparameter analysis of the apoptotic pathway in the samples of interest.


**References.**

1. Stennicke, H.T. and G.S. Salvesen. 1997. Biochemical characteristics of Caspases-3, -6, -7, and -8. *J. Biol. Chem*. 272: 25719-25723.
2. Nicholson, D.W., A. Ali, N.A. Thornberry, J.P. Vaillancourt, C.K. Ding, M. Gallant, Y. Gareau, P.R. Griffin, M. Labelle and M. Lazebnik. 1995. Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. *Nature* 376:37-43.
3. Patel, T., G.J. Gores and S.H. Kaufmann. 1996. The role of proteases during apoptosis. *FASEB J.* 10:587-597.

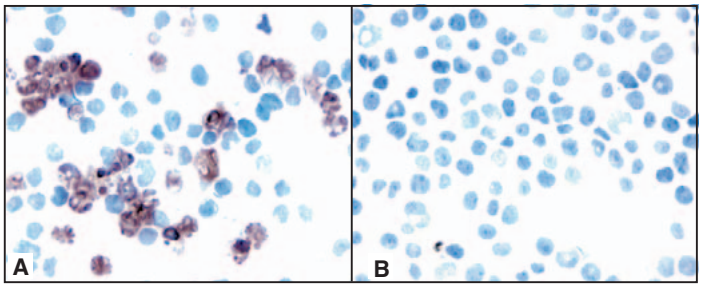




***Figure 4. Staining of human Jurkat T cells using an active caspase-3 polyclonal antibody.*** *Jurkat cells treated with 6 µM camptothecin for 4 hr to induce apoptosis (A) or untreated (B) were cytospinned and then acetone-fixed. Fixed cells were then stained with biotinylated active caspase-3 antibodies (Cat. No. 557038) and detected with a Streptavidin-Horseradish Peroxidase (HRP)-conjugated second step reagent.*

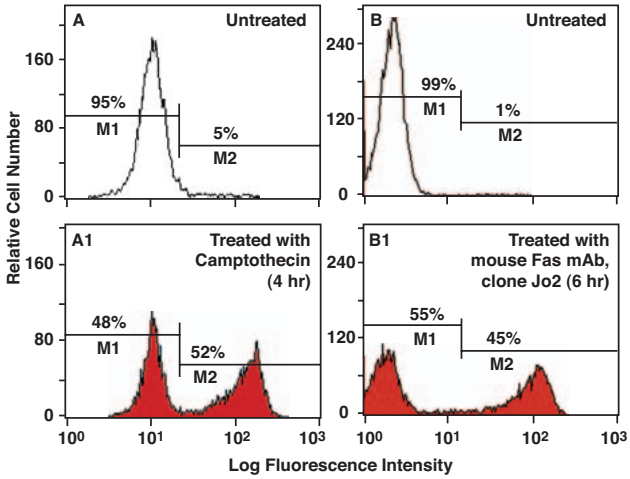



***Figure 5. Flow cytometric analysis of apoptotic and non-apoptotic populations using anti-active caspase-3 antibodies.*** *Jurkat T cells (A, A1) or mouse thymocytes (B, B1) were left untreated (A, B) or treated for 4 hr with camptothecin (A1) or a mouse Fas monoclonal antibody, clone Jo2 (Cat. No. 554254) to induce apoptosis (B1). Cells were permeabilized and then stained with PE-conjugated active caspase-3 antibodies (Cat. No. 557091). Untreated cells were primarily negative for the presence of active-caspase-3, whereas about half of each population of cells induced to undergo apoptosis had detectable active caspase-3.*

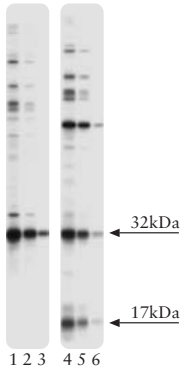



***Figure 6. Western blot analysis of caspase-3.*** *Lysates from control (lanes 1 – 3) and camptothecin-treated Jurkat cells (lanes 4 – 6) were probed with anti-caspase-3 (component 51-9000064) at the following dilutions: 1:1000 (lanes 1, 4), 1:2000 (lanes 2, 5), and 1:4000 (lanes 3, 6). caspase-3 is identified as an ~32 kDa band in control and treated cells and as an ~17 kDa band in treated cells.*

# Apoptosis

## Caspase-3 and Other Caspases

| DESCRIPTION | CLONE | FORMAT | ISOTYPE | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|---|---|
| **Apaf-1** | Polyclonal | Serum | Rab Ig | Hu | WB | 100 µl | **552813** |
| **Apaf-1** | 24 | Purified | Ms $IgG_1$ | Hu | WB, IF | 50/150 µg | **611364/65** |
| **Apaf-1** | Polyclonal | Purified | Rab Ig | Hu, Ms | WB | 50 µg | **559683** |
| **Caspase-2 (Ich-1L)** | 35 | Purified | Ms $IgG_1$ | Hu | IF, WB | 50/150 µg | **611022/23** |
| **Caspase-2 (Ich-1L)** | G310-1248 | Purified | Ms $IgG_1$ | Hu | WB | 50/150 µg | **551093/94** |
| **Caspase-2 (Ich-1L)** | Polyclonal | Serum | Rab Ig | Hu, Ms, Rat | WB | 100 µl | **552786** |
| **Caspase-3 (CPP32)** | 19 | Purified | Ms $IgG_{2a}$ | Hu | IF, IP, WB | 50/150 µg | **610322/23** |
| | 19 | HRP | Ms $IgG_{2a}$ | Hu | WB | 50/150 µg | **610324/25** |
| **Caspase-3 (CPP32)** | 46 | Purified | Ms $IgG_1$ | Ms | IF, WB | 50/150 µg | **611048/49** |
| **Caspase-3 (CPP32)** | Poly1324 | Serum | Rab Ig | Chick, Hu, Monk, Rat | WB | 100 µl | **552037** |
| **Caspase-3 (CPP32)** | Poly1325 | Serum | Rab Ig | Hu | WB | 100 µl | **552785** |
| **Caspase-3, Active Form (CPP32)** | C92-605 | Purified | Rab Ig | Hu, Ms | FCM, IHC(Fr), IP | 25 µg | **559565** |
| | C92-605 | Biotin | Rab Ig | Hu, Ms | FCM | 100 tests | **550557** |
| | C92-605 | FITC | Rab Ig | Hu, Ms | FCM | 100 tests | **559341** |
| | C92-605 | PE | Rab Ig | Hu | FCM | 100 tests | **550821** |
| **Caspase-3, Active Form (CPP32)** | Polyclonal | Purified | Rab Ig | Hu, Ms | FCM, IHC(Fr), IP | 0.1 mg | **557035** |
| | Polyclonal | Biotin | Rab Ig | Hu, Ms | IHC(Fr) | 0.1 mg | **557038** |
| | Polyclonal | PE | Rab Ig | Hu, Ms | FCM | 100 tests | **557091** |
| **Caspase-3, Active Form (CPP32)** | Polyclonal CM1 | Serum | Rab | Ms, Hu | WB | 100 µl | **551150** |
| **Caspase-3, Active Form, mAb Apoptosis Kit : PE (CPP32)** | C92-605 | Kit | Rab Ig | Hu, Ms | FCM | 100 tests | **550914** |
| **Pro-Caspase-3** | H15-575 | Purified | Ms $IgG_1$ | Hu | WB | 100 µg | **558611** |
| **Caspase-4 (TX, Ich-2)** | B25-1 | Purified | Ms $IgG_1$ | Hu, Ms, Rat | WB | 0.1 mg | **556459** |
| **Caspase-6 (Mch2a)** | B93-4 | Purified | Ms $IgG_1$ | Hu | WB | 0.1 mg | **556581** |
| **Caspase-7 (Mch3)** | 10-1-62 | Purified | Ms $IgG_1$ | Hu, Ms | IP, WB | 50/150 µg | **551238/39** |
| **Caspase-7 (Mch3)** | 11-1-56 | Purified | Ms $IgG_1$ | Hu | IP, WB | 50/150 µg | **551240/41** |
| **Caspase-7 (Mch3)** | 51 | Purified | Ms $IgG_{2b}$ | Dog, Hu | IF, WB | 50/150 µg | **610812/13** |
| **Caspase-7 (Mch3)** | 8-1-47 | Purified | Ms $IgG_1$ | Hu | IP, WB | 50/150 µg | **551236/37** |
| **Caspase-7 (Mch3)** | B94-1 | Purified | Ms $IgG_1$ | Hu | IP, WB | 0.1 mg | **556541** |
| **Caspase-7 (Mch3)** | Polyclonal | Serum | Rab Ig | Hu | WB | 100 µl | **552815** |
| **Caspase-8 (FLICE, MACH-1, Mch5)** | 3-1-9 | Purified | Ms $IgG_1$ | Hu | IP, WB | 50/150 µg | **551242** |
| **Caspase-8 (FLICE, MACH-1, Mch5)** | 4-1-20 | Purified | Ms $IgG_1$ | Hu | WB | 50/150 µg | **551244** |
| **Caspase-8 (FLICE, MACH-1, Mch5)** | B9-2 | Purified | Ms $IgG_{2a}$ | Hu | WB | 0.1 mg | **556466** |
| **Caspase-8 (FLICE, MACH-1, Mch5)** | Polyclonal | Serum | Rab Ig | Hu, Ms | WB | 100 µl | **559932** |
| **Caspase-8 (FLICE, MACH-1, Mch5)** | Polyclonal | Serum | Rab Ig | Hu | WB | 100 µl | **551234** |
| **Caspase-8 (FLICE, MACH-1, Mch5)** | Polyclonal | Serum | Rab Ig | Hu, Ms | WB | 100 µl | **552038** |
| **Caspase-9 (ICE-LAP-6, Mch6, Apaf-3)** | 2-22 | Purified | Ms $IgG_1$ | Hu | WB | 50/150 µg | **551246** |
| **Caspase-9 (ICE-LAP-6, Mch6, Apaf-3)** | B40 | Purified | Ms $IgG_1$ | Hu | WB | 0.1 mg | **556510** |
| **Caspase-9 (ICE-LAP-6, Mch6, Apaf-3)** | Polyclonal | Serum | Rab Ig | Hu, Ms | WB | 100 µl | **552036** |
| **Caspase-9 (ICE-LAP-6, Mch6, Apaf-3)** | Polyclonal | Serum | Rab Ig | Hu | IP, WB | 0.1 ml | **556585** |
| **Caspase-9 (ICE-LAP-6, Mch6, Apaf-3)** | Polyclonal | Purified | Rab Ig | Hu | WB | 50 µg | **550437** |
| **Caspase-9 (ICE-LAP-6, Mch6, Apaf-3)** | Polyclonal | Purified | Rab Ig | Hu | WB | 50 µg | **550438** |
| **Caspase-10** | Poly 1331 | Serum | Rab | Hu | WB | 100 µl | **552810** |
| **Caspase-10/b (FLICE2)** | Polyclonal | Purified | Rab Ig | Hu | IP, WB | 50 µg | **550742** |
| **Caspase-10/b (FLICE2)** | Polyclonal | Purified | Rab Ig | Hu | WB | 50 µg | **556564** |
| **Caspase-12** | Polyclonal | Purified | Rab Ig | Ms, Rat, Hu | WB | 100 µl | **551430** |
| **Caspase-13** | Polyclonal | Purified | Rab Ig | Ms, Rat, Hu | WB | 100 µl | **551443** |
| **Caspase-14 (mini-ICE, MICE)** | 32 | Purified | Ms $IgG_1$ | Hu, Ms | IF, WB | 50/150 µg | **611510** |
| **Caspase-14 (mini-ICE, MICE)** | 70A1426 | Purified | Ms $IgG_1$ | Hu, Ms | WB | 50/150 µg | **550872** |
| **c-IAP-1** | B75-1 | Purified | Ms $IgG_1$ | Hu | IP, WB | 0.1 mg | **556533** |
| **c-IAP-2** | F30-2285 | Purified | Ms $IgG_1$ | Hu | WB | 50 µg | **552782** |
| **CrmA** | A71-1 | Purified | Ms $IgG_1$ | VTC | WB | 0.1 mg | **556427** |
| **Cytochrome C** | 6H2.B4 | Purified | Ms $IgG_1$ | Hu, Ms, Rat | IP | 0.1 mg | **556432** |
| **Cytochrome C** | 7H8.2C12 | Purified | Ms $IgG_{2b}$ | Hu, Ms, Rat, Hs, Pg | WB | 0.1 mg | **556433** |
| **Hsp60** | 24 | Purified | Ms $IgG_1$ | Hu, Ms, Rat | IF, WB | 50/150 µg | **611562** |
| | 24 | FITC | Ms $IgG_1$ | Hu, Ms, Rat | IF | 50/150 µg | **611958** |
| **I-FLICE** | Polyclonal | Purified | Rab Ig | Hu | WB | 50 µg | **556567** |
| **Nedd4** | 15 | Purified | Ms $IgG_1$ | Ms | IF, WB | 50/150 µg | **611480** |
| **Nedd4** | Polyclonal | Serum | Rab | Hu, Ms | WB | 0.1 ml | **550598** |

## BD™ Cytometric Bead Array (CBA) for Multiplexed Quantitative Analysis of Caspase-3, PARP, and Bcl-2

- Convenient 96-well format for flow cytometry
- Fast, sensitive, multiplexed

BD™ Cytometric Bead Array (CBA) human apoptosis kit (Cat. No. 557816) is a bead based assay for rapid semi-quantification of active caspase-3, cleaved PARP and Bcl-2 in cell lysates by flow cytometry. The presence of active caspase-3 and cleaved PARP are indicative of apoptosis. The BD Cytometric Bead Array (CBA) human apoptosis Kit combines the principles of a sandwich ELISA with particle-based flow cytometry to analyze a cell lysate in a multiplexed assay. In this assay, cell lysates are incubated with beads. Each bead provides a capture surface for a specific protein and is analogous to an individually coated well in an ELISA plate. Fluorescently-labeled detection antibodies are added to the bead mixture with captured proteins and levels of active caspase-3, cleaved PARP, and Bcl-2 are measured by fluorescent intensity using a flow cytometer.

The kit also contains lyophilized apoptotic cell lysate for generating standard curves, which enables relative quantitation of active caspase-3, cleaved PARP, and Bcl-2 in the experimental cell lysate samples. Typically, detection of active caspase-3, cleaved PARP, and Bcl-2 in cell lysates have been analyzed by classical immunoprecipitation and Western blot technologies, which provides only semi-quantitative results. The BD CBA Human Apoptosis Kit offers an alternative to both conventional immunoprecipitation and Western blot analysis. BD CBA is capable of measuring caspase-3, PARP, and Bcl-2 over a two log dynamic range using a simple 4-hour assay protocol. The BD CBA technology has comparable analytical sensitivity and a wider dynamic range than conventional ELISA. Compared to immunoprecipitation and Western blot techniques, BD CBA is more quantitative, requires less sample, takes less time, allows analysis of multiple proteins, and is more amenable to high throughput screening.

For detailed protocols, consult the manual or visit **www.bdbiosciences.com/pharmingen/cba**

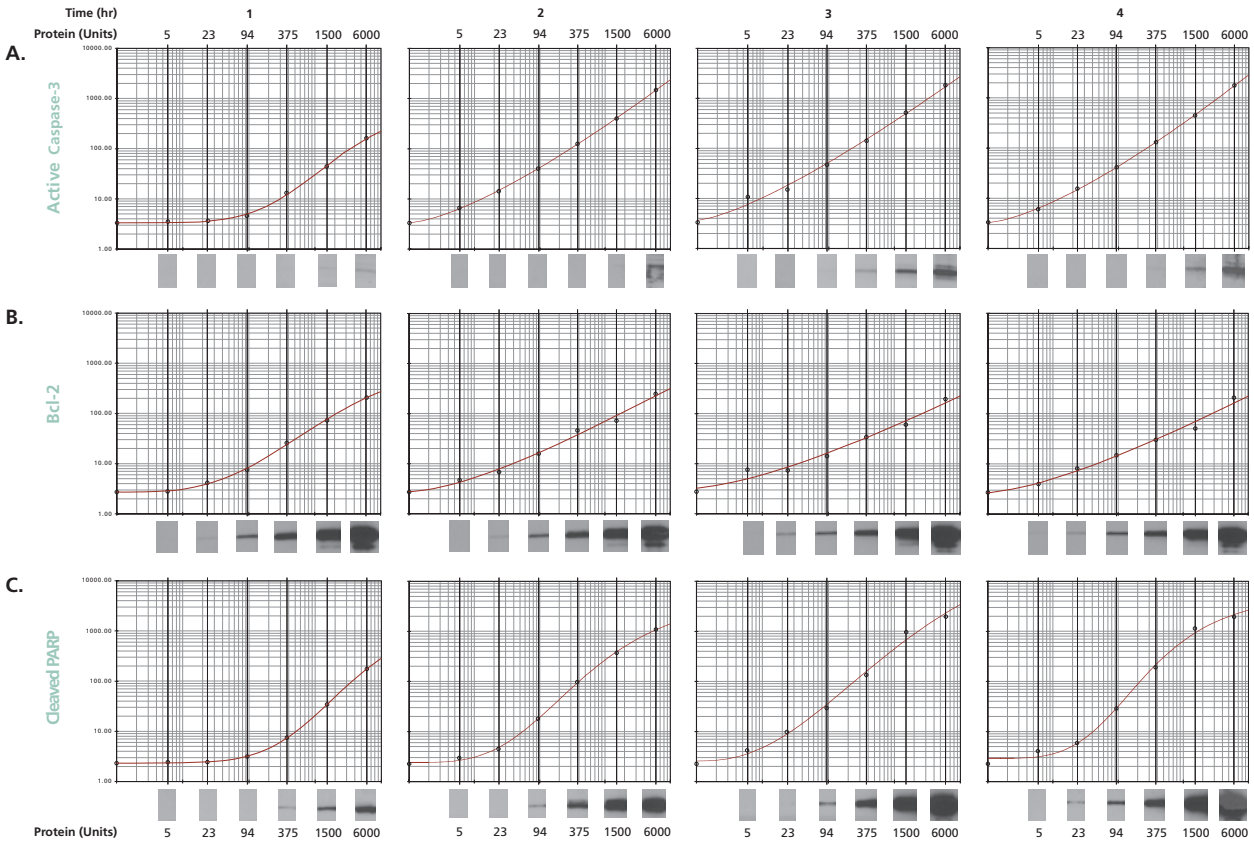



***Figure 7. Time course analysis of active caspase-3, Bcl-2, and cleaved PARP protein levels in Jurkat T Cells.*** *Cells were treated with 4µM camptothecin for 1 hr, 2 hr, 3 hr, or 4 hr to induce apoptosis. Cells were washed twice with ice-cold PBS, lysed with lysis buffer and analyzed for levels of active caspase-3 (A), Bcl-2 (B), and cleaved PARP (C) using the BD™ CBA Human Apoptosis Kit. Protein levels are expressed in Units. A unit of active caspase-3, Bcl-2, or cleaved PARP corresponds to the amount of active caspase-3, Bcl-2, or cleaved PARP protein in 0.1µg of total protein from camptothecin-treated Jurkat cell lysate. In addition, parallel samples were tested by Western blot (A, B, C). (A): caspase-3 pAb (Cat. No. 552785), (B) Bcl-2 mAb (clone Bcl-2/100, Cat. No. 556354), and (C) cleavage-specific PARP mAb (clone F21-852, Cat. No. 552596) were used for Western blot.*

| DESCRIPTION | FORMAT | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|
| **BD CBA Human Apoptosis Kit** | (Cleaved PARP, Bcl-2, Active Caspase-3) | Hu | FCM | 50 tests | **557816** |

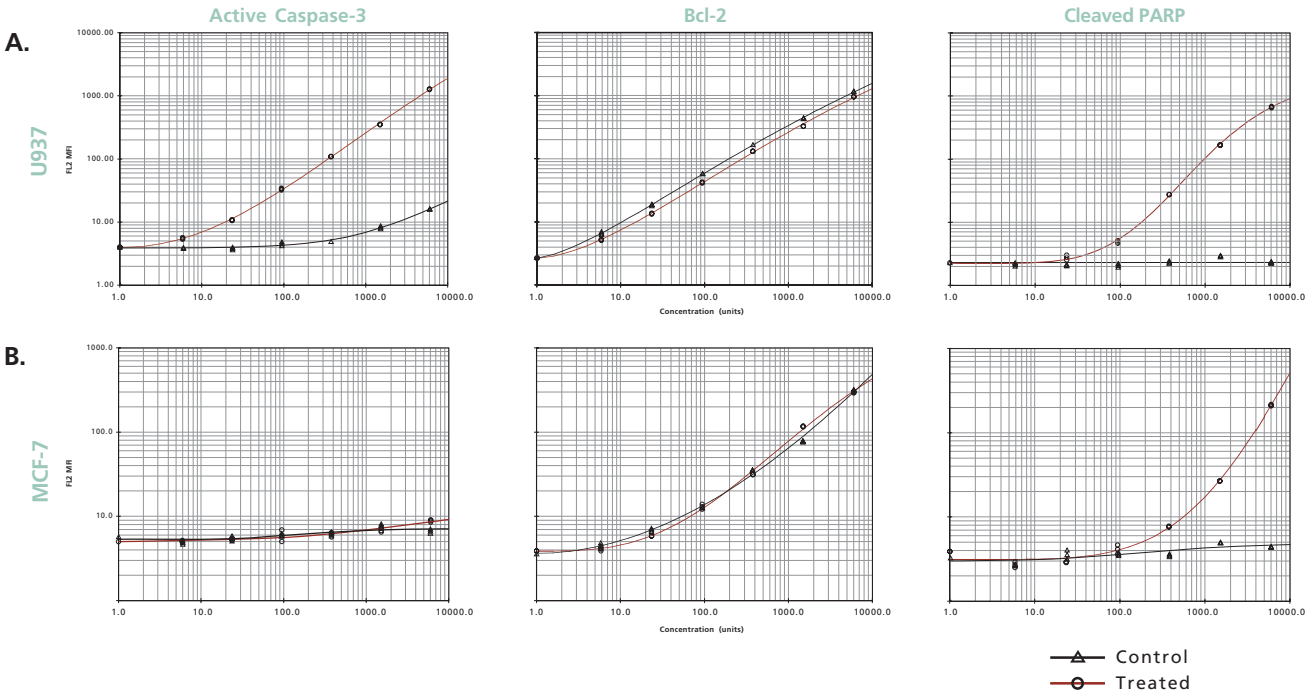



***Figure 8. Active caspase-3, Bcl-2, and cleaved PARP levels in U937 and MCF-7 Cells.*** *U937 (A) and MCF-7 (B) cells were either untreated (control) or treated with 4µM camptothecin for 4 hr to induce apoptosis. Cells were washed twice with ice-cold PBS, lysed with lysis buffer, and analyzed for levels of active Caspase-3, Bcl-2, and cleaved PARP using a novel, sensitive, and reproducible BD™ CBA Apoptosis kit. Protein levels are expressed in units.*

## Active Caspase-3 ELISA

BD Biosciences – Pharmingen has developed an ELISA assay for detecting active caspase-3 which enables high-throughput screening for the detection of apoptosis. ELISA assays performed on Jurkat T cells induced to undergo apoptosis with camptothecin or staurosporine indicate that active caspase-3 was detected in lysates from treated cells but not from control (untreated) cells. More active caspase-3 was detected in lysates from staurosporine-treated cells than from camptothecin-treated cells, indicating that different treatments induce different levels of active caspase-3. Taken together, the results indicate that our new active caspase-3 ELISA assay is specific for detection of active caspase-3 in apoptotic cells. This fast and specific assay is useful for screening a large number of samples for the presence of active caspase-3, and expands our comprehensive offering of state-of-the art reagents for studying apoptosis.

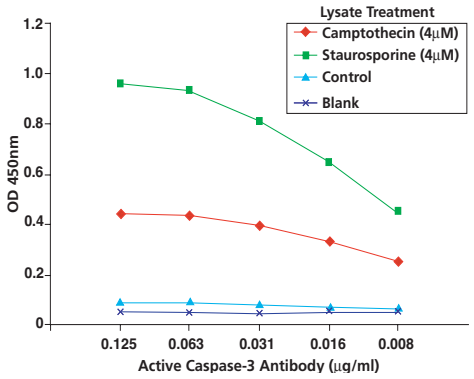



***Figure 9. Active caspase-3 expression in camptothecin- and staurosporine-treated Jurkat cells.*** *Jurkat cells were left untreated (control) or treated with 4 µM camptothecin (4 hr) or 4 µM staurosporine (18 hr) to induce apoptosis. Active caspase-3 was detected in apoptotic, but not in control cells. The level of active caspase-3 in staurosporine-treated Jurkat lysate was 2.5 times greater than in camptothecin- treated Jurkat lysate. The capture antibody was used at 1.0 µg/ml and the detection antibody was serially diluted to 0.008 µg/ml.*

| DESCRIPTION | CLONE | FORMAT | ISOTYPE | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|---|---|
| **Caspase-3, Active Form, ELISA Pair (CPP32)** | | Purified | Rab Ig | Hu | ELISA | Reagents for 10 Plates | **550578** |
| **Apoptotic Jurkat ELISA Standard** | | | | | ELISA | 500 µg | **550943** |
| **BD OptEIA Active Caspase-3 ELISA Set** | | Set | | Hu | ELISA | Reagents for 5 Plates | **inquire** |
| **BD OptEIA Cleaved PARP ELISA Set** | | Set | | Hu | ELISA | Reagents for 5 Plates | **inquire** |

## Caspase Activity Assays: Kits and Reagents

- Profile multiple caspases *in vitro*
- Fast, quantitative, sensitive, and easy spectrofluorometric assay
- Convenient 96-well format

**Active caspase enzymes:** Caspases are cysteine proteases synthesized as inactive proenzymes. Caspases, expressed in *E. coli,* spontaneously undergo autoprocessing to yield the characteristics of the active enzymes. These active, recombinant forms of human caspase-3, -6, -7, and -8 provide researchers with an additional tool for characterization of apoptotic pathways by allowing differentiation of caspases involved in selected models of apoptosis. Using different peptide substrates, the level of activity of multiple caspases can be estimated and compared. Many caspases have the ability to cleave the same substrate, at least *in vitro*, although with different efficiency. It should be noted that the synthetic substrates are the only preferred substrate motif, and cleavage is dependent on factors such as pH, concentration, reaction time, and accessibility.

**Caspase inhibitors and substrates:** Synthetic tetra-peptide sequences chosen for use as substrates or inhibitors mimic the cleavage site of the various caspase enzymes. These substrates are cleaved between aspartate, D, and AFC/AMC*, releasing the fluorogenic molecule AFC or AMC which may be detected by ultraviolet (UV) spectrofluorometry. The released AFC molecule can be monitored by a blue to green shift in fluorescence (excitation, 400 nm; emission, 505 nm). Likewise, the cleavage of AMC can be monitored with excitation, 360 nm and emission at 450 nm. Spectrofluorometric detection of released fluorogenic molecules can be used for high-throughput detection of apoptosis in lysed cells and tissue.

When coupled to an aldehyde group (CHO), these tetra-peptides function as potent inhibitors of caspase activity and can be used to block caspase-mediated cleavage of the corresponding fluorogenic substrates for the specific caspase involved in cell death.

BD Biosciences offers a range of tools for caspase activity assays from individual fluorogenic peptide substrates and inhibitors, to kits, to ready-to-use assay plates. All are based on the use of synthetic tetrapeptide substrates that are designed such that proteolytic cleavage by active human caspases results in release of a fluorophore or chromophore.

The individual synthetic tetra-peptide substrates, together with the caspase inhibitors and active caspase enzymes, offer flexibility in the experimental design of a caspase activity assay.

For detailed information on the contents of the kits and assay protocol, please refer to Technical Data Sheet or User Manual for individual products.

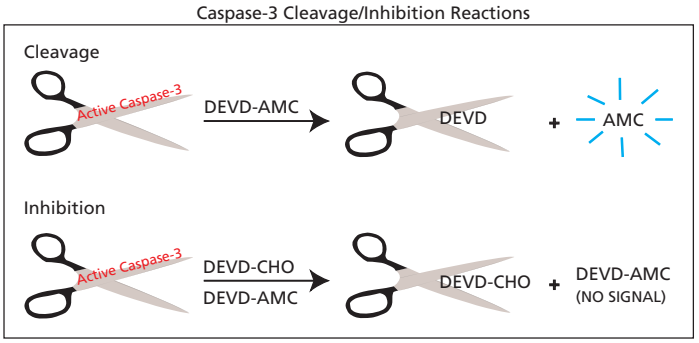



***Figure 10. Caspase-3 Fluorogenic Assay.*** *Active caspase-3 binds to the fluorogenic Ac-DEVD-AMC substrate and cleaves it between asparatic acid (D) and AMC, releasing the fluorescent AMC. AMC fluorescence is quantified by UV spectrofluorometry. The Ac-DEVD-CHO aldehyde inhibitor binds strongly to the caspase-3 active site and blocks substrate binding. Hence, Ac-DEVD-AMC is not cleaved and fluorescence is not emitted.*

**7-amino-4-trifluoromethyl coumarin (AFC), 7- amino-4-methylcoumarin (AMC)*

# Apoptosis

## Peptides

| DESCRIPTION | FORMAT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|
| Caspase-1 Fluorogenic Substrate, Ac-YVAD-AMC | Purified | SF, FA | 1 mg | 556451 |
| Caspase-3 Fluorogenic Substrate, Ac-DEVD-AFC | Purified | SF, FA | 1 mg | 556574 |
| Caspase-3 Fluorogenic Substrate, Ac-DEVD-AMC | Purified | SF, FA | 1 mg | 556449 |
| Caspase-3 Inhibitor, Ac-DEVD-CHO | Purified | SF, FA | 1 mg | 556465 |
| Caspase-3 Inhibitor, Z-DEVD-FMK | Purified | FA, SF | 1 mg | 550378 |
| Caspase-6 Fluorogenic Substrate, Ac-VEID-AFC | Purified | SF, FA | 1 mg | 556548 |
| Caspase-6 Inhibitor, Ac-VEID-CHO | Purified | SF, FA | 1 mg | 556550 |
| Caspase-6 Inhibitor, Z-VEID-FMK | Purified | FA, SF | 1 mg | 550379 |
| Caspase-8 Fluorogenic Substrate, Ac-IETD-AFC | Purified | SF, FA | 1 mg | 556552 |
| Caspase-8 Inhibitor, Ac-IETD-CHO | Purified | SF, FA | 1 mg | 556554 |
| Caspase-8 Inhibitor, Z-IETD-FMK | Purified | FA, SF | 1 mg | 550380 |
| Caspase-8, Active Form, Recombinant Human Protein (FLICE) | Purified | FA, SF | 5 µg | 556481 |
| Caspase-9 Inhibitor, Z-LEHD-FMK | Purified | FA, SF | 1 mg | 550381 |
| General Caspase Inhibitor, Z-VAD-FMK | Purified | FA, SF | 1.0 mg | 550377 |
| Caspase Inhibitor Negative Control, Z-FA-FMK | Purified | FA, SF | 1 mg | 550411 |

## BD Pharmingen™ Caspase Assay Sets

| DESCRIPTION | FORMAT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|
| Caspase-3, Active Form, Recombinant Human Protein (CPP32) | Purified | FA, SF | 10 µg | 556471 |
| Caspase-3, Active Form, Recombinant Human Protein (CPP32) | Purified | FA, SF | 5 µg | 556472 |
| Caspase-6, Active Form, Recombinant Human Protein (Mch2) | Purified | FA, SF | 5 µg | 556475 |
| Caspase-6, Active Form, Recombinant Human Protein (Mch2) | Purified | FA, SF | 10 µg | 556474 |
| Caspase-7, Active Form, Recombinant Human Protein (Mch3) | Purified | FA | 10 µg | 556477 |
| Caspase-7, Active Form, Recombinant Human Protein (Mch3) | Purified | FA, SF | 5 µg | 556478 |
| Caspase-8, Active Form, Recombinant Human Protein (FLICE) | Purified | FA, SF | 10 µg | 556480 |

## Recombinant Proteins

| DESCRIPTION | FORMAT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|
| Caspase-3, Active Form, Enzyme Set | Set | FA, SF | 20 tests | 556473 |
| Caspase-7, Active Form, Enzyme Set | Set | FA, SF | 20 tests | 556479 |
| Caspase-8, Active Form, Enzyme Set | Set | FA, SF | 20 tests | 556482 |

## QTL Lightspeed™ Enzyme Activity Assays

QTL Lightspeed™ protease activity assays are a universal mix and measure assay for protease activity that does not require antibodies or radioactive labels. The QTL Lightspeed™ technology combines high sensitivity with high speed for high-throughput screening of kinases, based on QTL's patented fluorescence "superquenching" technology (see below).

### The main features of this assay are:

- Homogeneous one-step "mix and measure" process
- No washing steps
- No radioactive compounds
- No antibodies
- No custom instrumentation
- Works with either peptide or protein substrates
- Validated for miniaturization: 96, 384, 1536, and 3456-well plate formats
- Highly robust (Z′ ≥ 0.8; Z′ at 10% conversion ≥ 0.6)
- Excellent sensitivity and speed

The QTL approach to biosensing takes advantage of superquenching of fluorescent polymers by electron and energy transfer quenchers. This assay platform utilizes the light harvesting ability of conjugated polymers along with their highly delocalized excited state to provide highly amplified fluorescent signal modulation in response to the presence of very small quantities of electron and energy transfer species. This novel technology has been applied to the highly sensitive detection of proteins, small molecules, peptides, proteases, kinases, and oligonucleotides by associating the signal modulation phenomenon with antigen-receptor, substrate-enzyme, and oligo-oligo binding interactions. The assay can be run using either 96, 384, 1536, or 3456-well plates. When used with the calibrator peptide (included and specific for each protease assay) in the endpoint measurement kit, the researcher is able to generate standard curves and determine the amount of sample phosphorylation.

The QTL Lightspeed Protease Activity Assays can be used in virtually all commercially-available fluorescence spectrometers and multi-well plate readers. The excitation (blue) and detection (green) wavelengths are compatible with all common fluorimeters.

### QTL Lightspeed™ Protease assay: How it works (see figure 11)

- Detection system consists of a fluorescent polymer and quencher-tether-ligand (QTL) moieties
- The QTL moieties consist of a quencher that quenches polymer fluorescence, a ligand that is specifically cleaved by the target enzyme, and a tether that connects the quencher and ligand
- When the reactive tether is cleaved by enzyme, the quencher is released from the polymer, resulting in a quantitative signal that increases with enzyme activity or duration of the assay

### QTL Lightspeed™ kinase and phosphatase assays

Using the same technology, QTL also offers kinase and phosphatase activity assays.

Certain assay development is available with short turnaround times.

QTL BIOSYSTEMS

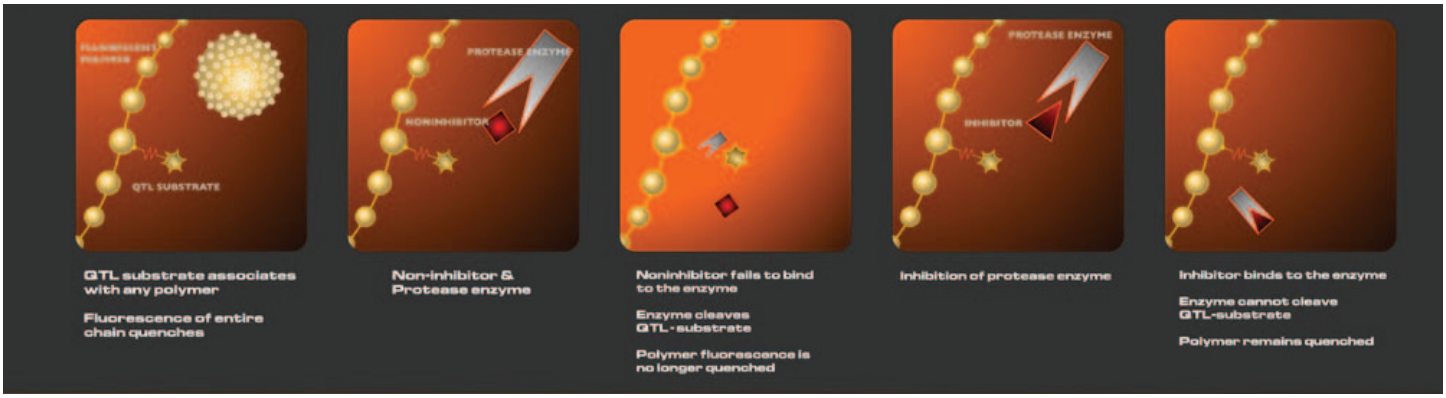



*Figure 11. QTL Lightspeed™ protease assay: How it works*

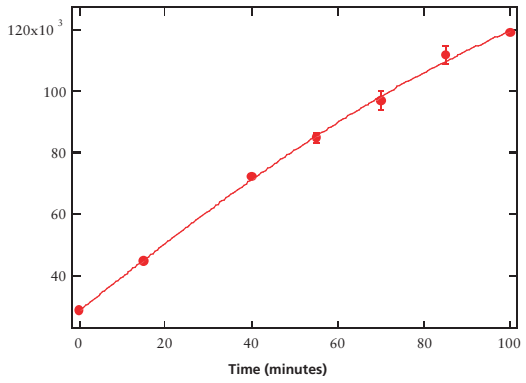



*Figure 12. QTL Lightspeed™ caspase-3 enzyme kinetics. The assay was carried out in a total volume of 10 µL with 1.5 µM substrate and 30 pg of caspase-3 (purchased from a commercial source). Enzyme and substrate were diluted using Assay Buffer. Reactions were carried out in a 384-well white plate at ambient temperature (~25°C) by mixing 5 µL enzyme and 5 µL substrate at various time intervals. The plate was agitated for 30 sec using the auto mix function of the microplate reader. Reactions were carried out under yellow light to minimize exposure to white light. Reactions were terminated by the addition of 40 µL of QTL Sensor to each reaction well. The plate was agitated after the addition of QTL Sensor. Reaction progression was monitored by measuring the emission $\lambda_{em}$ at 576 nm with a $\lambda_{co}$ 475 nm cut-off filter, after exciting the samples at $\lambda_{ex}$ 440 n*

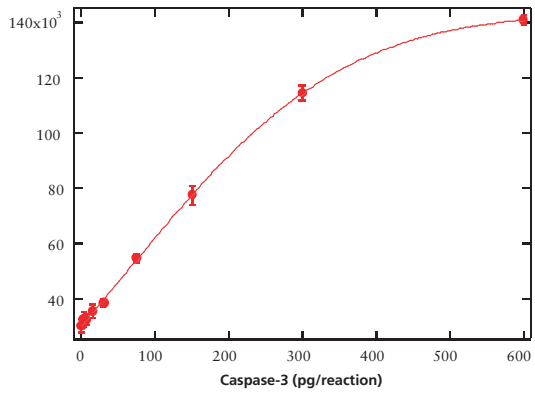



*Figure 13. QTL Lightspeed™ caspase-3 concentration curve – limit of detection (LOD). The QTL Lightspeed™ caspase-3 assay was carried out in a total volume of 10 µL containing 1.5 µM QTL substrate and increasing concentrations of caspase-3, both diluted using Assay Buffer. Reactions were terminated after 60 minutes by the addition of 40 µL QTL Sensor.*

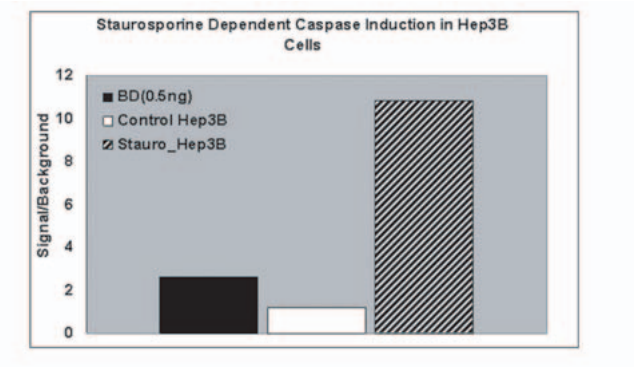



*Figure 14. QTL Lightspeed™ caspase-3/7 detection of staurosporine induced caspase-3/7 activity in Hep3B cell lysate. The QTL Lightspeed™ caspase-3 assay was carried out on Hep3B cell lysate treated with 10 µM Staurosporine for 6 hours to induce apoptosis. The results (patterned bar) are compared to signal observed with 0.5 ng of purified caspase-3 (black solid bar) and to signal observed with Hep3B cells not treated with Staurosporine (white solid bar). The QTL Lightspeed™ caspase-3 assay was carried out in a total volume of 10 µL containing 10 µM substrate diluted using Assay Buffer. Reactions were terminated after 60 minutes by the addition of 40 µL QTL Sensor.*

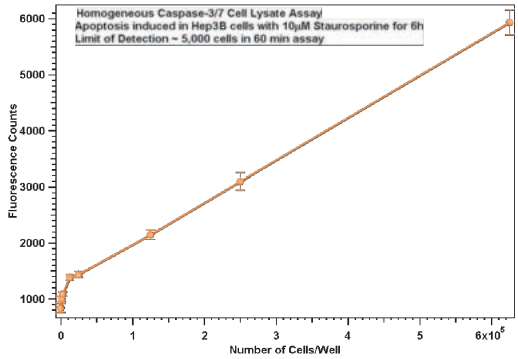



*Figure 15. QTL Lightspeed™ caspase-3/7 activity detection in cell lysate-limit of detection (LOD). The QTL Lightspeed™ caspase-3 assay was carried out on increasing amounts of Hep3B cell lysate treated with 10 µM Staurosporine for 6 hours to induce apoptosis. The assay was conducted in a total volume of 10 µL containing 10 µM diluted using Assay Buffer. Reactions were terminated after 60 minutes by the addition of 40 µL QTL Sensor. Other reaction conditions are as listed under Figure 12.*

| DESCRIPTION | FORMAT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|
| Caspase-3/7 | kit | FA, HTS | 100 tests | **558172** |
| | kit | FA, HTS | 400 test | **558173** |

## Membrane Permeable Caspase Inhibitors

- Cell-based assays
- Block activity of single or multiple caspases

The discovery that activation of apoptotic caspases is a cellular death sentence has led researchers to explore strategies to block caspase activation. Caspase inhibitors are emerging as a key anti-apoptotic tool, and treatment with these inhibitors prior to apoptotic-inducing events has been shown to reduce cell death or enhance survival in various model systems.

The fluoromethyl ketone (FMK) inhibitors are designed to be used in both *in vivo* and *in vitro* cell-based assays to measure inhibition of apoptosis. Membrane permeable caspase inhibitors would allow researchers to determine whether apoptosis is induced through the mitochondria, through caspase activation pathways, or through the activation of cell death receptors. The FMK-based caspase inhibitors are cell-permeable due to esterification of aspartic or glutamic acid at the carboxyl group. Esterification makes inhibitors more hydrophobic. The amino end of the inhibitor is modified with N-benzyloxycarbonyl (Z) which increases hydrophobicity of the molecule and makes Z-inhibitors more cell-permeable. These inhibitors covalently modify the thiol group of the caspase enzymes making them irreversible inhibitors. Membrane permeable inhibitors can be used as general caspase inhibitor or as inhibitors of specific caspase activity to study events downstream of activation of specific caspases.

## BD™ APOBlock

BD™ APOBlock is a membrane permeable cell culture-grade inhibitor that can be used to investigate the effects of irreversibly blocking caspase activity while maintaining sterility.

BD APOBlock is a proprietary peptidomimetic compound that acts as a broad-spectrum caspase inhibitor. It can be used as a sterile media supplement to inhibit apoptosis and promote cell survival, cell preservation, and recovery from cryogenic storage.

BD APOBlock may also allow users to separate caspase activity from other cell death pathways.

| DESCRIPTION | APPS | SIZE | CAT. NO. |
|---|---|---|---|
| **Caspase-3 Inhibitor, Z-DEVD-FMK** | FA, SF | 1 mg | **550378** |
| **Caspase-6 Inhibitor, Z-VEID-FMK** | FA, SF | 1 mg | **550379** |
| **Caspase-8 Inhibitor, Z-IETD-FMK** | FA, SF | 1 mg | **550380** |
| **Caspase-9 Inhibitor, Z-LEHD-FMK** | FA, SF | 1 mg | **550381** |
| **General Caspase Inhibitor, Z-VAD-FMK** | FA, SF | 1.0 mg | **550377** |
| **Caspase Inhibitors Negative Control, Z-FA-FMK** | FA, SF | 1 mg | **550411** |
| **BD ApoBlock Caspase Inhibitor** | FA, SF, FCM | 1.0 mg | **552892** |

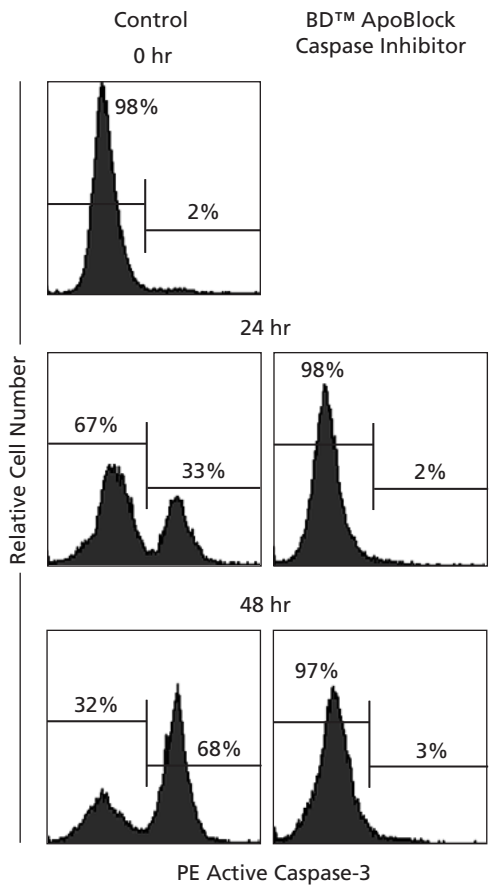



*Figure 16. Time course analysis of active caspase-3 expression. Freshly isolated mouse thymocytes were either left untreated or were treated with a BD™ ApoBlock caspase inhibitor (40mm final concentration) for 24 hr or 48 hr and stained with PE anti-active caspase-3 Ab (Cat. No. 550914, clone C92-605). Flow cytometry was performed on a BD FACSCalibur™ flow cytometer (BD Biosciences, San Jose, CA).*

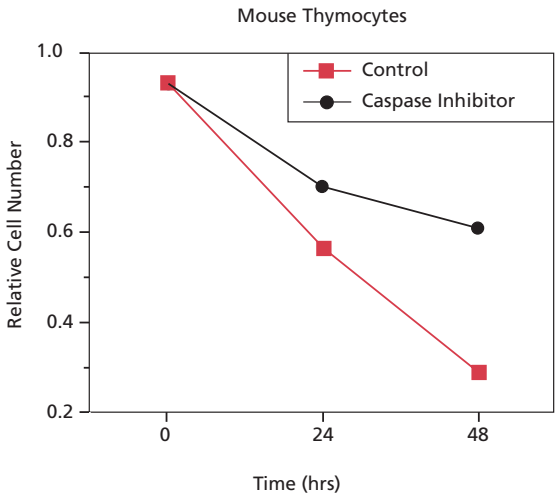



*Figure 17. Inhibition of cell death using BD™ ApoBlock caspase inhibitor. Cells were treated as described in Figure 14. Cell viability was determined by Trypan blue exclusion and cells were counted using a hemacytometer. There was an overall reduction of relative cell number in both cultures over the time course. However, after 48 hours, approximately 90% of the untreated population had died, while more than 60% of the population treated with caspase inhibitor was still alive.*

# Apoptosis

## Analysis of Caspase Cleavage Products

- Western blot
- Immunohistochemistry
- Immunoprecipitation
- Flow cytometry

Apoptosis signaling that occurs through the cascade of caspases activation results in extensive downstream cleavage of protein substrates, leading to a loss of cellular structure and function which culminates in cell death. Patterns of protein cleavage provide insight into the mechanisms of cellular processes and cell death. For example, the ~116 kDa nuclear enzyme PARP is typically cleaved into fragments of ~85 kDa and 24 kDa by active caspase-3 during apoptosis.[1]

During necrosis, a 50 kDa fragment has been observed that is caspase-3 independent and appears to result from cleavage by lysosomal proteins.[2] BD Biosciences – Pharmingen has antibodies which detect cleavage of PARP and are suitable for Western blot, flow cytometry and inmmunofluorescence applications. The increasing availability of antibodies recognizing protein cleavage fragments should help elucidate the relationships between cleavage patterns and mechanisms involved in cellular processes such as apoptosis and necrosis.

**References**


1. Alexandre, S., C. Rast, G. Nguyen-BA, G.G. Poierier, and P. Vasseur. 2000. PARP degradation in apoptotic Syrian hamster embryo (SHE) cells compared to HL60 cell line. *Biochimie* 82:1115-1122.
2. Gobeil, S., C.C. Boucher, D. Nadeau, G.G. Poierier. 2001. Characterization of the necrotic cleavage of poly(ADP-ribose) polymerase (PARP-1) implication of lysosomal proteases. *Cell Death Differ*. 8:588-94.

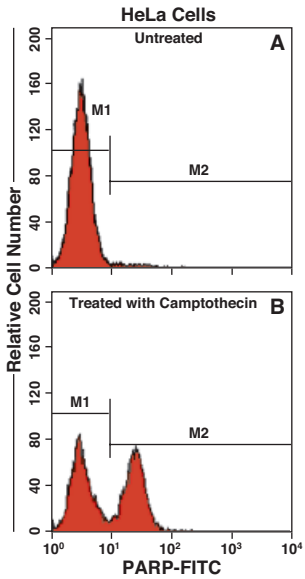




***Figure 18. Flow cytometric analysis of cleaved PARP.*** *Jurkat T cells were either left untreated (A) or treated with camptothecin (4 mm, 4 hr) to induce apoptosis (B). Cells were fixed and permeabilized for 30 min at RT (BD Cytofix/Cytoperm, Cat. No. 554714) and stained with PARP-FITC (Cat.No. 551528, 2 µl/1x10⁶ cells) for 1 hr at RT. Cells were then washed twice with Perm/Wash buffer (component of Cat. No. 554714), resuspended in Perm/Wash Buffer and analyzed by flow cytometry. The results indicate that untreated cells were primarily negative for cleaved PARP (A, M1), whereas about 40% of the apoptotic cell population was positive for cleaved PARP (B, M2).*

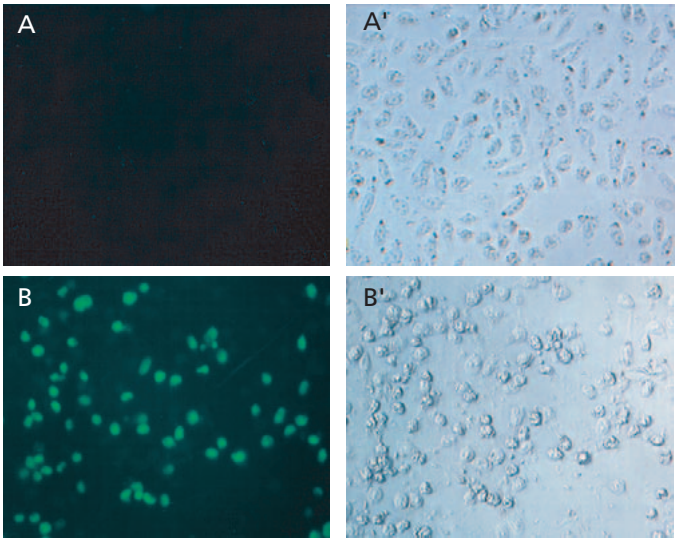



***Figure 19. Immunofluorescence of cleaved PARP.*** *Hela cells growing on Labtek II chambered slides were either left untreated (A) or treated with staurosporine (1.0 mM, 4 hr) to induce apoptosis (B). Cells were then fixed with 3.7% formaldehyde (15 min on ice), then permeabilized in 0.25% Triton X-100/3% BSA/PBS (15 min on ice). Cells were then washed twice with 3% BSA/PBS and stained with 4 µl/ml of FITC-labeled rabbit anti-PARP (Cat. No. 551528) in 3% BSA/PBS (1 hr at RT). Cells were washed twice with 3% BSA/PBS and then visualized by immunofluorescence microscopy. A' and B' represent phase correlates of A and B, respectively. The results indicate that untreated cells were primarily negative for cleaved PARP (A); whereas, a significant percentage of the staurosporine-treated population is positive for cleaved PARP.*

## Analysis of Caspase Cleavage Products *(continued)*

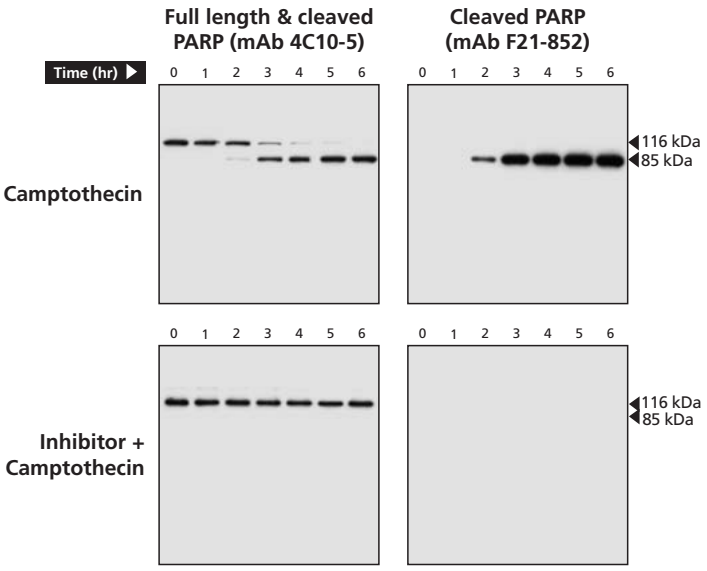



***Figure 20. Western blot analysis of PARP in camptothecin-treated HL-60 cells in the presence and absence of a broad spectrum caspase inhibitor (Z-VAD-FMK).*** *HL-60 cell cultures were treated with camptothecin alone or pre-incubated with Z-VAD-FMK (Cat. No. 550377) for 30 min before adding camptothecin. Cells were lysed at various times over a 6 hr time course, and equal amounts of total cell proteins were separated by SDS-PAGE and blotted with two different PARP monoclonal antibodies. Clone 4C10-5 (Cat. No. 556494) recognizes both the 116 kDa intact form of PARP and the 85 kDa cleavage fragment. Clone F21-852 (Cat. No. 552596/97) is specific for the 85 kDa PARP cleavage fragment.**

*Clone 4C10-5 identified PARP as a 116 kDa band in both the presence and absence of Z-VAD-FMK. Both antibodies identified an 85 kDa band in cultures that lacked Z-VAD-FMK. This band was observed at 2 hr and beyond, and correlated with a progressive loss of the 116 kDa form identified by clone 4C10-5. The 85 kDa form was not identified by either antibody in cultures containing Z-VAD-FMK indicating that PARP cleavage was blocked in these cultures. These results illustrate the cleavage-specific nature of clone F21-852 for the 85 kDa PARP fragment.*

**Note: Camptothecin is a topoisomerase I inhibitor that is widely used to induce apoptosis. The 85 kDa fragment results from a specific pattern of PARP cleavage by active caspase-3 and is considered to be a hallmark of apoptosis. Z-VAD-FMK blocks caspase activity, and hence the formation of the 85 kDa PARP fragment.*

## Antibodies to Caspase-3 Protein Substrate

| DESCRIPTION | CLONE | FORMAT | ISOTYPE | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|---|---|
| **D4-GDI** | Polyclonal | Serum | Rab | Hu | WB | 0.1 ml | **556498** |
| **D4-GDI (Cleavage-Specific)** | 97A1015 | Purified | Ms $IgG_1$ | Ms, Hu | WB | 50 µg | **550746** |
| **D4-GDI (Cleavage-Specific)** | 97A1015 | PE | Ms $IgG_1$ | Ms, Hu | FCM | 50 µg | **551546** |
| **D4-GDI (Cleavage-Specific)** | 97A1015 | FITC | Ms $IgG_1$ | Ms, Hu | FCM | 50 µg | **551547** |
| **DFF (N-Terminal)** | Polyclonal | Purified | Rab Ig | Hu | WB | 50 µg | **556545** |
| **DNA-PK (p350) (p350)** | 4F10C5 | Purified | Ms $IgG_1$ | Mam | IP, WB | 0.1 mg | **556456** |
| **Fractin (Cleaved Actin)** | Polyclonal | Purified | Rab Ig | Hu | IHC, WB | 100 µl | **551527** |
| **PARP** | 42 | Purified | Ms $IgG_1$ | Dog, Hu, Ms, Rat | IF, WB | 50/150 µg | **611038/39** |
| **PARP** | C2-10 | Ascites | Ms $IgG_1$ | Hu, Ms, Ham, Bov | IF, WB | 0.1 ml | **556362** |
| **PARP** | 4C10-5 | Purified | Ms $IgG_1$ | Hu | Blot, FCM, IP, WB | 0.1 mg | **556494** |
| **PARP** | 7D3-6 | Purified | Ms $IgG_1$ | Hu | WB | 50/150 µg | **551024/25** |
| **PARP, Cleavage Site-Specific** | Polyclonal | FITC | Rab | Hu, Bov | FCM, IF | 200 µl | **551528** |
| **PARP, Cleavage Site-Specific** | Poly1318 | Serum | Rab Ig | Bov, Hu | WB | 200 µl | **551923** |
| **PARP, Cleaved Form (Asp214)** | F21-852 | Purified | Ms $IgG_1$ | Hu, Ms | WB | 50/150 µg | **552596/07** |
| **PKC** | MC5 | Purified | Ms $IgG_{2a}$ | Hu, Ms, Rat, Bov, Chick, Rab | IF, IHC(Fr), IP, WB | 0.1 mg | **554207** |
| **PRK2 (PKN2, PAK-2)** | 22 | Purified | Ms $IgG_1$ | Hu | IF, WB | 50/150 µg | **610794/95** |
| **Rb (a.a. 332-344)** | G3-245 | Purified | Ms $IgG_1$ | Ms, Rat, Hu, Monk, Qua, Mink | FCM, GS, IF, IHC(F), IHC(Fr), IP, WB | 0.1 mg | **554136** |
| **RhoGDI/D4-GDI** | Polyclonal | Serum | Rab | Hu | WB, IP | 0.1 ml | **556511** |
| **SREBP-1** | IgG-2A4 | Purified | Ms $IgG_1$ | Hu, Ham | IP, WB | 0.1 mg | **557036** |
| **SREBP-2** | IgG-1C6 | Purified | Ms $IgG_1$ | Hu | IP, WB | 0.1 mg | **557037** |

## BD OptEIA Sets

| DESCRIPTION | FORMAT | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|
| **BD OptEIA Cleaved PARP ELISA Set** | Set | Hu | ELISA | Reagents for 5 Plates | **inquire** |

## DNA Fragmentation

- Detect nuclear DNA fragmentation in conjunction with cell cycle analyses
- Can be used with adherent cells or suspension cells
- Analyze results by flow cytometry

One of the later steps in apoptosis is DNA fragmentation, a process which results from the activation of endonucleases during the apoptotic program.[1, 2] These nucleases degrade the higher order chromatin structure into fragments of ~300 kb and subsequently into smaller DNA pieces of about 50 bp in length.[3] A method often used to detect fragmented DNA utilizes a reaction catalyzed by exogenous terminal transferase, TdT, often referred to as "end-labeling" or "TUNEL" (terminal deoxynucleotidyltransferase dUTP nick end labeling).[4] APO-BrdU and Apo-Direct kits detect DNA fragmentation in adherent cells and in cells growing in suspension using flow cytometry in conjunction with cell cycle analysis.

### APO-BRDU™ Assay

In this assay, TdT catalyzes a template-independent addition of bromolated deoxyuridine triphosphates (Br-dUTP) to the 3′-hydroxyl (OH) termini of double- and single-stranded DNA.[5] After incorporation, these sites are identified by flow cytometry by staining cells with FITC-labeled anti-BrdU.

### The APO-DIRECT™ Assay

This assay provides a single-step method for labeling DNA breaks with FITC-dUTP, followed by flow cytometric analysis.[6]

Flow cytometry allows simultaneous analysis for several parameters, including size, granularity, and different fluorescent labeling of the same cells.

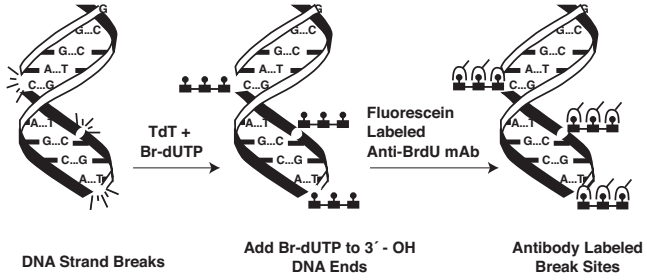



*Figure 21. Schematic Representation of APO-BRDU™ Labeling. The enzyme deoxynucleotidyl transferase (TdT) catalyzes a template independent addition of bromolated deoxyuridine triphosphates (Br-dUTP) to the 3′-hydroxyl (-OH) ends of double- and single-stranded DNA. After Br-dUTP incorporation, DNA break sites are identified by a FITC-labeled anti-BrdU mAb.*

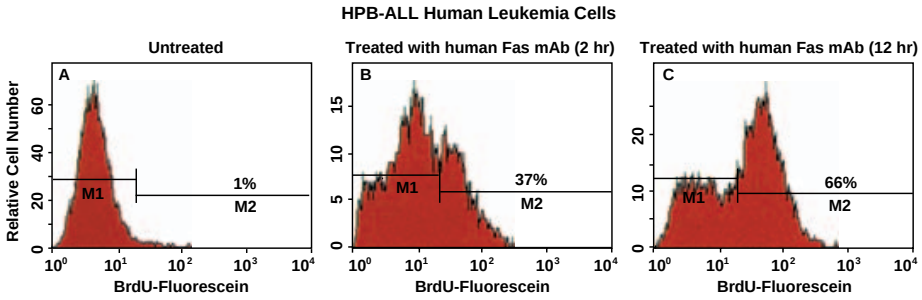



*Figure 22. Flow Cytometric Analysis of HPB-ALL Human Leukemia Cells Using APO-BrdU™. HPB-ALL human leukemia cells were left untreated (A) or treated with anti-human Fas mAb, clone DX2 (Cat. No. 555670) and Protein G for 2 hr (B) or 12 hr (C). Cells were fixed and incubated with Br-dUTP in the presence of TdT enzyme in order to incorporate Br-dUTP into exposed 3′-OH DNA ends. Br-dUTP was detected with a fluorescein labeled anti-BrdU mAb. Non-apoptotic cells (M1 gates) do not incorporate significant amounts of Br-dUTP due to lack of exposed 3′-OH ends, and consequently have relatively little fluorescence compared to apoptotic cells which have an abundance of 3′-OH ends (M2 gates). DX2-induced, Fas-mediated apoptosis is shown by increases in the number of cells staining with anti-BrdU-Fluorescein mAb (M2 gates) after 2 and 12 hr. The M1 and M2 gates demarcate non-apoptotic and apoptotic populations, respectively.*

| DESCRIPTION | FORMAT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|
| **APO-DIRECT Apoptosis Detection Kit** | Kit | FCM | 50 tests | **556381** |
| **APO-BRDU Apoptosis Detection Kit** | Kit | FCM | 60 tests | **556405** |

**Reference**


1. Enari, M., H. Sakahira, H. Yokoyama, K. Okawa, A. Iwamatsu, and S. Nagata.1998. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature* 391:43-50.
2. Sakahira, H., M. Enari, and S. Nagata. 1998. Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. *Nature* 391:96-99.
3. Walker, R.P., L. Kokileva, J. LeBlanc and M. Sikorska. 1993. Detection of the initial stages of DNA fragmentation in apoptosis. *Biotechniques* 15:1032-1036.
4. Darzynkiewicz, Z., G. Juan, X. Li, W. Gorczyca, T. Murakami, and T. Traganos, T. 1997. Cytometry in cell necrobiology: Analysis of apoptosis and accidental cell death (necrosis). *Cytometry* 27:1-20.
5. Li, X. and A. Darzynkiewicz. 1995. Labeling DNA strand breaks with Brd-UTP. Detection of apoptosis and cell proliferation. *Cell Prolif.* 28: 572-579.
6. Li, X., F. Traganos, M.R. Melamed, and Z. Darzynkiewicz.1995. Single step procedure for labeling DNA strand breaks with fluorescein- or BODIPY-conjugated deoxynucleotides. Detection of apoptosis and bromodeoxyuridine incorporation. *Cytometry* 20:172-180.

## Individual Antibodies to Proteins Involved in Apoptosis

### Bcl-2 Family

Members of the Bcl-2 family play a major role in regulating the response of cells to a wide variety of apoptotic signals. Several of these proteins act as inhibitors of apoptosis, whereas others promote cell death. It is thought that protein-protein interactions between Bcl-2 family members are an important factor in their role in apoptosis.

### Bcl-2 Family

| DESCRIPTION | CLONE | FORMAT | ISOTYPE | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|---|---|
| **Bad** | 48 | Purified | Ms $IgG_{2b}$ | Hu, Ms, Rat | IF, IHC, WB | 50/150 µg | **610391/32** |
| | 48 | FITC | Ms $IgG_{2b}$ | Hu, Ms, Rat | IF | 50/150 µg | **610393/94** |
| | 48 | TRITC | Ms $IgG_{2b}$ | Hu, Ms, Rat | IF | 50/150 µg | **610395/96** |
| **BAG-1** | 19 | Purified | Ms $IgG_1$ | Hu | WB | 50/150 µg | **611868/69** |
| **Bak** | G317-2 | Purified | Ms $IgG_1$ | Hu | WB | 0.1 mg | **556382** |
| | G317-1 | Purified | Ms IgM | Hu | IHC(Fr) | 0.1 mg | **556384** |
| **Bak** | Polyclonal | Serum | Rab | Hu, Ms, Rat | IHC(P), WB | 0.1 ml | **556396** |
| **Bax** | 3 | Purified | Ms $IgG_1$ | Dog, Hu | IF, IP, WB | 50/150 µg | **610982/83** |
| **Bax** | 6A7 | Purified | Ms $IgG_1$ | Hu, Ms, Rat | IP, WB | 0.1 mg | **556467** |
| **Bax** | G206-1276 | Purified | Rat IgM | Hu, Ms | IHC(F), IHC(Fr), IP, WB | 0.1 mg | **554082** |
| **Bax** | Polyclonal | Serum | Rab | Ms, Rat | IHC(F), IHC(Fr), IP, WB | 0.1 ml | **554106** |
| **Bax** | Polyclonal | Serum | Rab | Hu | IHC(F), IHC(Fr), IP, WB | 0.1 ml | **554104** |
| **Bcl-10** | 151 | Purified | Ms $IgG_1$, κ | Hu | FCM | 0.1 mg | **551340** |
| **Bcl-2** | 100 | FITC | Ms $IgG_1$ | Hu | IC, FCM | 50 tests | **340575** |
| | 100 | PE | Ms $IgG_1$ | Hu | IC, FCM | 50 tests | **340576** |
| **Bcl-2** | 3F11 | Purified | Ar Ham IgG | Ms | FCM, IF, IHC(Fr), IP, WB | 0.1 mg | **554218** |
| | 3F11 | FITC Set | Ar Ham IgG | Ms | FCM | 100 tests | **554221** |
| | 3F11 | PE Set | Ar Ham IgG | Ms | FCM | 100 tests | **556537** |
| **Bcl-2** | 4D7 | Purified | Ms $IgG_1$ | Hu | EM, IP, Oth, WB | 50/150 µg | **551097/98** |
| **Bcl-2** | 6C8 | Purified | Ar Ham IgG | Hu | WB | 50/150 µg | **551051/52** |
| | 6C8 | FITC Set | Ar Ham IgG | Hu | FCM | 100 tests | **554234** |
| | 6C8 | PE Set | Ar Ham IgG | Hu | FCM | 100 tests | **556536** |
| **Bcl-2** | 7 | Purified | Ms $IgG_1$ | Chick, Dog, Hu, Ms, Rat | IF, IHC, IP, WB | 50/150 µg | **610538/39** |
| **Bcl-2** | Bcl-2/100 | Purified | Ms $IgG_1$ | Hu | FCM, IHC(Fr), WB, IHC(P) | 50/150 µg | **551107/09** |
| | Bcl-2/100 | Purified | Ms $IgG_1$ | Hu | IHC(Fr), IHC(F)* | 1 ml | **550847** |
| | Bcl-2/100 | FITC Set | Ms $IgG_1$ | Hu | FCM | 100 tests | **556357** |
| | Bcl-2/100 | PE Set | Ms $IgG_1$ | Hu | FCM | 100 tests | **556535** |
| **Bcl-2** | Polyclonal | Serum | Rab | Ms, Rat | IHC(F), IHC(Fr), IP, WB | 0.1 ml | **554087** |
| **Bcl-2** | Polyclonal | Serum | Rab | Ms, Rat | IHC(Fr), IHC(P), IP, WB | 0.1 ml | **554279** |
| **Bcl-2** | Polyclonal | Serum | Rab | Hu | IF, IHC(F), IHC(Fr), IP, WB | 0.1 ml | **554160** |
| **Bcl-w** | 16H12 | Purified | Rat $IgG_{2a}$ | Ms, Hu | WB | 50 µg | **550559** |
| **Bcl-x** | 2H12 | Purified | Ms $IgG_{2a}$ | Hu, Ms | WB | 50/150 µg | **551020/22** |
| **Bcl-x** | 2H12 | Purified | Ms $IgG_{2a}$ | Hu, Ms, Rat | WB | 0.1 mg | **556499** |
| **Bcl-x** | 4 | Purified | Ms $IgG_{2b}$ | Chick, Ms, Rat | IF, IHC, WB | 50/150 µg | **610209/10** |
| **Bcl-x** | 44 | Purified | Ms $IgG_1$ | Hu, Ms, Rat | IF, IHC, WB | 50/150 µg | **610746/47** |
| **Bcl-x** | Polyclonal | Purified | Rab Ig | Chick, Dog, Hu, Ms, Rat | IF, IHC, WB | 50/150 µg | **610211/12** |
| **Bcl-x** | Polyclonal | Serum | Rab | Hu, Ms | IHC(F), IHC(Fr), WB | 100 µl | **551269** |
| **Bcl-xL/S** | Polyclonal | Serum | Rab | Hu, Ms | IHC(F), IHC(Fr), IHC(P), WB | 0.1 ml | **556361** |
| **Beclin** | 20 | Purified | Ms $IgG_{2a}$ | Ms, Rat, Hu, Dog, Chick | WB | 50/150 µg | **612112/13** |
| **Bid** | 40 | Purified | Ms $IgG_1$ | Ms | WB | 50/150 µg | **611866/67** |
| **Bid** | 7 | Purified | Ms $IgG_1$ | Hu | IF, WB | 50/150 µg | **611528/29** |
| **Bid** | Polyclonal | Serum | Rab | Hu | IP, WB | 0.1 ml | **550365** |
| **Bid** | Polyclonal | Serum | Rab | Hu, Ms | WB | 0.1 ml | **559681** |
| **Nip1** | 5 | Purified | Ms $IgG_1$ | Hu, Rat | IF, WB | 50 µg | **611096** |
| **Bcl-2 Related Sampler Kit** contains: Bad, BAG-1, Bax, Bcl-2, Bcl-x, Becin, Bid | | Purified | | | WB | 10 µg each | **612742** |

# Apoptosis

## Signaling Molecules

Apoptosis is one of a number of phenotypic responses mediated by signal transduction pathways occurring in the cell. Clustering of cellular receptors is a commonly observed first step in the mechanism of signal transduction pathways which results in apoptosis. Clustered receptor cytoplasmic domains trigger subsequent steps in signal transduction pathways. An important link in this system is provided by signal molecules which bind directly to the intracellular domains of receptors or within a receptor signal complex. Within the TNF receptor family (TNFR types 1 and 2, Fas, TRAIL-R1 through -R4), many proteins fall into one of two categories based on structural considerations: the so called "death domain" homologues and the TNFR associated factors (TRAFs). These proteins share sequence motifs which facilitate protein-protein interaction(s) with receptors and other signal proteins within the receptor complex. Thus, these proteins provide an important early step in the signal transduction pathways that trigger apoptosis.

## Death Receptors and Ligands

| DESCRIPTION | CLONE | FORMAT | ISOTYPE | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|---|---|
| **CD120a (TNF Receptor Type I)** | MABTNFR1-A1 | Purified | Ms $IgG_1$ | Hu | ELISA Cap. | 0.5 mg | **552535** |
| | MABTNFR1-B1 | Purified | Ms $IgG_{2a}$, κ | Hu | FCM | 0.5 mg | **550514** |
| | MABTNFR1-B1 | Biotin | Ms $IgG_{2a}$, κ | Hu | FCM | 0.5 mg | **550900** |
| | MABTNFR1-B1 | Biotin | Ms $IgG_{2a}$, κ | Hu | FCM, IF, ELISA Det. | 0.5 mg | **552536** |
| **CD120a (TNF Receptor, Type I/p55)** | 55R-286 | Purified | Ar Ham IgG1, l 1 | Ms | FCM | 0.5 mg | **559915** |
| | 55R-593 | NA/LE | Ar Ham IgG1, κ | Ms | Block | 0.25 mg | **557536** |
| | 55R-170 | NA/LE | Ar Ham IgG1, κ | Ms | Block | 0.25 mg | **557535** |
| **CD120b (TNF Receptor Type II)** | MR2-2 | Purified | Ms $IgG_1$, κ | Hu | ELISA Cap. | 0.5 mg | **552478** |
| | hTNFR-M1 | Purified | Rat $IgG_{2b}$, κ | Hu | FCM | 0.5 mg | **551311** |
| | hTNFR-M1 | Biotin | Rat $IgG_{2b}$, κ | Hu | ELISA Det., FCM | 0.5 mg | **552477** |
| | hTNFR-M1 | Biotin | Rat $IgG_{2b}$, κ | Hu | FCM | 100 tests | **552417** |
| | hTNFR-M1 | PE | Rat $IgG_{2b}$, κ | Hu | FCM | 100 tests | **552418** |
| | hTNFR-M1 | APC | Rat $IgG_{2b}$, κ | Hu | FCM | 100 tests | **552419** |
| **CD120b (TNF Receptor, Type II/p75)** | TR75-89 | Purified | Ar Ham IgG1, l3 | Ms | FCM | 0.5 mg | **559916** |
| | TR75-89 | Biotin | Ar Ham IgG1, l3 | Ms | FCM | 0.5 mg | **550476** |
| | TR75-89 | PE | Ar Ham IgG1, l3 | Ms | FCM | 0.2 mg | **550086** |
| | TR75-32 | NA/LE | Ar Ham IgG1, l 1 | Ms | Block | 0.25 mg | **557533** |
| | TR75-54 | NA/LE | Ar Ham IgG1, l3 | Ms | Block | 0.25 mg | **557534** |
| **CD178 (Fas Ligand, CD95 Ligand)** | MFL3 | Purified | Ar Ham IgG1, κ | Ms | FCM | 0.5 mg | **555291** |
| | MFL3 | Biotin | Ar Ham IgG1, κ | Ms | FCM | 0.5 mg | **555292** |
| | MFL3 | PE | Ar Ham IgG1, κ | Ms | FCM | 0.2 mg | **555293** |
| | MFL3 | NA/LE | Ar Ham IgG1, κ | Ms | FCM | 0.5 mg | **555290** |
| **CD178 (Fas Ligand, CD95 Ligand)** | MFL4 | Purified | Ar Ham IgG3, κ | Ms, Rat | FCM | 0.5 mg | **555022** |
| | MFL4 | Biotin | Ar Ham IgG3, κ | Ms, Rat | FCM | 0.5 mg | **556998** |
| | MFL4 | NA/LE | Ar Ham IgG3, κ | Ms, Rat | FCM | 0.5 mg | **555021** |
| **CD178 (Fas Ligand, CD95 Ligand)** | NOK-1 | Purified | Ms $IgG_1$ | Hu | FCM | 100 µg | **556372** |
| | NOK-1 | Biotin | Ms $IgG_1$ | Hu | FCM | 100 µg | **556373** |
| | NOK-1 | Biotin | Ms $IgG_1$ | Hu | FCM | 100 tests | **556374** |
| | NOK-1 | NA/LE | Ms $IgG_1$ | Hu | FA, FCM | 0.25 mg | **556371** |
| | NOK-2 | Purified | Ms $IgG_{2a}$ | Hu | ELISA, FA, IP | 0.1 mg | **556376** |
| | NOK-2 | NA/LE | Ms $IgG_{2a}$ | Hu | ELISA, FA, IP | 0.25 mg | **556375** |
| | G247-4 | Purified | Ms $IgG_1$ | Hu | IHC(Fr), IP, WB | 0.1 mg | **556387** |
| | G247-4 | Purified | Ms $IgG_1$ | Hu | IHC(Fr), IHC(F)* | 1 ml | **550841** |
| **CD95 (Fas/APO-1)** | Jo2 | Purified | Ar Ham IgG2, l2 | Ms | FCM, IP | 500 µg | **554255** |
| | Jo2 | Biotin | Ar Ham IgG2, l2 | Ms | FCM | 0.5 mg | **554256** |
| | Jo2 | FITC | Ar Ham IgG2, l2 | Ms | FCM | 0.5 mg | **554257** |
| | Jo2 | PE | Ar Ham IgG2, l2 | Ms | FCM | 0.2 mg | **554258** |
| | Jo2 | Alexa Fluor® 647 | Ar Ham IgG2, l2 | Ms | FCM | 0.1 mg | **557693** |
| | Jo2 | Alexa Fluor® 488 | Ar Ham IgG2, l2 | Ms | FCM | 0.1 mg | **557679** |
| | Jo2 | PE-Cy7 | Ar Ham IgG2, l2 | Ms | FCM | 0.1 mg | **557653** |
| | Jo2 | NA/LE | Ar Ham IgG2, l2 | Ms | FCM, FA, IP | 0.5 mg | **554254** |

## Death Receptors and Ligands *(continued)*

| DESCRIPTION | CLONE | FORMAT | ISOTYPE | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|---|---|
| **CD95 (Fas/APO-1)** | DX2 | Purified | Ms $IgG_1$, κ | Hu | FCM, IHC(Fr) | 0.1 mg | **555671** |
| | DX2 | Biotin | Ms $IgG_1$, κ | Hu | FCM | 100 tests | **555672** |
| | DX2 | FITC | Ms $IgG_1$, κ | Hu | FCM | 100 tests | **555673** |
| | DX2 | PE | Ms $IgG_1$, κ | Hu | FCM | 100 tests | **555674** |
| | DX2 | APC | Ms $IgG_1$, κ | Hu | FCM | 100 tests | **558814** |
| | DX2 | PE-Cy5 | Ms $IgG_1$, κ | Hu | FCM | 100 tests | **559773** |
| | DX2 | NA/LE | Ms $IgG_1$, κ | Hu | FCM, FA | 0.5 mg | **555670** |
| | EOS9.1 | Purified | Ms IgM, κ | Hu | FCM, FA | 0.1 mg | **550042** |
| | G254-274 | Purified | Ms $IgG_1$ | Hu | WB | 0.1 mg | **556370** |
| **CD95 (Fas/APO-1)** | 13 | Purified | Ms $IgG_1$ | Chick, Dog, Hu, Ms, Rat | IF, IHC, WB | 50/150 µg | **610197/98** |
| **DcR1** | Polyclonal | Purified | Rab Ig | Hu | WB | 200 µl | **550622** |
| **DR3** | Polyclonal | Purified | Rab Ig | Hu | WB | 50 µg | **556566** |
| **DR4 (C-Terminal)** | Polyclonal | Purified | Rab Ig | Hu | WB | 50 µg | **556544** |
| **DR4 (N-Terminal)** | Polyclonal | Purified | Rab Ig | Hu | WB | 50 µg | **556543** |
| **DR6** | Polyclonal | Purified | Rab Ig | Hu | WB | 50 µg | **550439** |
| **DRAK2** | Polyclonal | Purified | Rab Ig | Hu | WB | 200 µl | **550945** |
| **FADD** | 1 | Purified | Ms IgG1 | Hu | IF, WB | 50 µg | **610399** |
| **FADD** | 1 | Purified | Ms $IgG_1$ | Hu | IF, WB | 150 µg | **610400** |
| **FADD** | A66-2 | Purified | Ms $IgG_1$ | Hu | IP, WB | 0.1 mg | **556402** |
| **Granzyme B** | 2CF/F5 | Purified | Ms $IgG_{2a}$ | Hu, Rat | WB | 50 µg | **550558** |
| | GB11 | FITC | Ms $IgG_1$, κ | Hu | FCM | 100 tests | **558132** |
| | GB11 | Alexa Fluor® 647 | Ms $IgG_1$, κ | Hu | IC/FCM | 0.1 mg | **557867** |
| | GB11 | Alexa Fluor® 700 | Ms $IgG_1$, κ | Hu | IC/FCM | 0.1 mg | **557971** |
| **LT-α** | 359-238-8 | Purified | Ms $IgG_1$ | Hu | ELISA Cap. | 1.0 mg | **551222** |
| **LT-α** | 359-81-11 | Purified | Ms $IgG_1$ | Hu | Block, IC/FCM | 0.1 mg | **554554** |
| **LT-α** | 359-81-11 | Biotin | Ms $IgG_1$ | Hu | ELISA Det. | 0.5 mg | **554555** |
| **LT-α** | 359-81-11 | PE | Ms $IgG_1$ | Hu | IC/FCM | 0.1 mg | **554556** |
| **LT-α** | 359-81-11 | NA/LE | Ms $IgG_1$ | Hu | Neu | 0.5 mg | **554553** |
| **Mcl-1** | 22 | Purified | Ms $IgG_1$ | Hu | IP, WB | 0.1 mg | **559027** |
| | Polyclonal | Serum | Rab | Hu | IHC(F), IHC(Fr), IP, WB | 0.1 ml | **554103** |
| **Perforin** | dG9 | Purified | Ms $IgG_{2b}$ | Hu | FCM, IHC(Fr), IP | 0.1 mg | **556434** |
| | dG9 | FITC Set | Ms $IgG_{2b}$ | Hu | FCM | 100 tests | **556577** |
| | dG9 | PE Set | Ms $IgG_{2b}$ | Hu | FCM | 100 tests | **556437** |
| **RIP** | 38 | Purified | Ms $IgG_{2a}$ | Chick, Dog, Hu, Ms, Rat | IF, IP, WB | 50 µg | **610458** |
| | 38 | Purified | Ms $IgG_{2a}$ | Chick, Dog, Hu, Ms, Rat | IF, IP, WB | 150 µg | **610459** |
| | G322-2 | Purified | Ms $IgG_1$ | Hu | WB | 50 µg | **551041** |
| | G322-2 | Purified | Ms IgG | Hu | WB | 150 µg | **551042** |
| | Polyclonal | Serum | Rab | Hu | WB | 0.1 ml | **559689** |
| **SODD** | Polyclonal | Serum | Rab | Hu | WB | 200 µl | **550857** |

# Apoptosis

## Death Receptors and Ligands *(continued)*

| DESCRIPTION | CLONE | FORMAT | ISOTYPE | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|---|---|
| **TNF** | MAb1 | Purified | Ms $IgG_1$ | Hu | ELISA Cap., WB | 1.0 mg | **551220** |
| | MAb11 | Purified | Ms $IgG_1$ | Hu | IC/FCM Block | 0.1 mg | **554510** |
| | MAb11 | Purified | Ms $IgG_1$ | Hu | ICC | 0.25 mg | **559071** |
| | MAb11 | Purified | Ms $IgG_1$ | Bab, Rhe, Cyno | IC/FCM | 0.1 mg | **558882** |
| | MAb11 | Biotin | Ms $IgG_1$ | Hu | ELISA Det. | 0.5 mg | **554511** |
| | MAb11 | FITC | Ms $IgG_1$ | Hu | IC/FCM | 0.1 mg | **554512** |
| | MAb11 | FITC | Ms $IgG_1$ | Bab, Cyno, Rhe | IC/FCM | 50 tests | **552889** |
| | MAb11 | PE | Ms $IgG_1$ | Hu | IC/FCM | 0.1 mg | **554513** |
| | MAb11 | PE | Ms $IgG_1$ | Hu | IC/FCM | 100 tests | **559321** |
| | MAb11 | PE | Ms $IgG_1$ | Bab, Rhe, Cyno | IC/FCM | 50 tests | **557068** |
| | MAb11 | APC | Ms $IgG_1$ | Hu | IC/FCM | 0.1 mg | **554514** |
| | MAb11 | APC | Ms $IgG_1$ | Bab, Cyno, Rhe | IC/FCM, FCM | 50 tests | **551384** |
| | MAb11 | Alexa Fluor® 488 | Ms $IgG_1$ | Hu | IC/FCM | 100 tests | **557722** |
| | MAb11 | Alexa Fluor® 647 | Ms $IgG_1$ | Hu | IC/FCM | 100 tests | **557733** |
| | MAb11 | PE-Cy7 | Ms $IgG_1$ | Hu | FCM | 100 tests | **557647** |
| | MAb1 | NA/LE | Ms $IgG_1$ | Hu | Neu | 0.5 mg | **554508** |
| **TNF** | MABTNF-A5 | NA/LE | Ms $IgG_{2a}$, κ | Hu | Neu | 0.5 mg | **552467** |
| **TNF** | MP6-XT22 | Purified | Rat $IgG_1$ | Ms | WB, IC/FCM Block, ELISA Cap | 0.1 mg | **554416** |
| | MP6-XT22 | Purified | Rat $IgG_1$ | Ms | ICC | 0.25 mg | **559064** |
| | MP6-XT22 | FITC | Rat $IgG_1$ | Ms | IC/FCM | 0.1 mg | **554418** |
| | MP6-XT22 | PE | Rat $IgG_1$ | Ms | IC/FCM | 0.1 mg | **554419** |
| | MP6-XT22 | APC | Rat $IgG_1$ | Ms | IC/FCM | 0.1 mg | **554420** |
| | MP6-XT22 | Alexa Fluor® 488 | Rat $IgG_1$ | Ms | IC/FCM | 0.1 mg | **557719** |
| | MP6-XT22 | Alexa Fluor® 647 | Rat $IgG_1$ | Ms | IC/FCM | 0.1 mg | **557730** |
| | MP6-XT22 | PE-Cy7 | Rat $IgG_1$ | Ms | IC/FCM | 0.1 mg | **557644** |
| **TNF** | MP6-XT3 | Biotin | Rat $IgG_1$ | Ms | ELISA Det., WB | 0.5 mg | **554415** |
| | MP6-XT3 | NA/LE | Rat $IgG_1$ | Ms | Neu | 0.5 mg | **554414** |
| **TNF** | Polyclonal | Purified | Gt IgG | Rab | ELISA Cap. | 1.0 mg | **551214** |
| | Polyclonal | Biotin | Gt IgG | Rab | ELISA Det. | 1.0 mg | **551213** |
| | Polyclonal | NA/LE | Gt IgG | Rab | FA | 0.5 mg | **553629** |
| **TNF** | TN3-19.12 | Purified | Ar Ham IgG1, l 1 | Rat, Ms | Block, IC/FCM | 0.1 mg | **559500** |
| | TN3-19.12 | Purified | Ar Ham IgG1, l 1 | Rat, Ms | ELISA Cap., IC/FCM Block, IP, WB | 0.5 mg | **557516** |
| | TN3-19.12 | PE | Ar Ham IgG1, l 1 | Rat, Ms | IC/FCM | 0.1 mg | **559503** |
| | TN3-19.12 | NA/LE | Ar Ham IgG1, l 1 | Ms, Rat | Neu | 0.5 mg | **557370** |
| **TNF** | G281-2626 | Purified | Rat $IgG_1$ | Ms | WB, ELISA Cap. | 1.0 mg | **551225** |
| | G281-2626 | NA/LE | Rat $IgG_1$ | Ms | Neu | 0.5 mg | **554640** |
| **TNF** | 23H1.1 | Biotin | Ms $IgG_1$, κ | Rab | ELISA Det. | 0.5 mg | **552470** |
| **TNF** | C1150-14 | Biotin | Rab Ig | Rat, Ms | ELISA Det. | 0.5 mg | **557432** |
| **TRADD** | 37 | Purified | Ms $IgG_1$ | Hu, Rat | IF, IHC, IP, WB | 50/150 µg | **610572/73** |
| **TRADD** | B36-2 | Purified | Ms $IgG_1$ | Hu | WB | 0.1 mg | **556496** |
| **TRAF3** | B1-6 | Purified | Ms $IgG_1$ | Hu | WB | 0.1 mg | **556461** |
| **TRAIL** | B35-1 | Purified | Ms $IgG_{2b}$ | Hu | WB | 0.1 mg | **556468** |
| | N2B2 | Purified | Rat $IgG_{2a}$, κ | Ms | FCM | 0.1 mg | **550320** |
| | RIK-1 | Purified | Ms $IgG_1$ | Hu | FCM | 0.1 mg | **550517** |
| **TRAIL** | RIK-2 | Purified | Ms $IgG_1$ | Hu | FCM | 0.1 mg | **550515** |
| | RIK-2 | Biotin | Ms $IgG_1$ | Hu | FCM | 200 µg | **550431** |
| | RIK-2 | PE | Ms $IgG_1$ | Hu | FCM | 0.1 mg | **550516** |
| | RIK-2 | NA/LE | Ms $IgG_1$ | Hu | FA | 250 µg | **550912** |
| **TRAIL-R2 (DR5)** | MD5-1 | Purified | Ar Ham $IgG_2$, κ | Ms | FCM, FA | 0.1 mg | **557868** |

## Death Receptors and Ligands *(continued)*

*Fast Immune and Multicolor Cocktails*

| DESCRIPTION | CLONE | FORMAT | ISOTYPE | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|---|---|
| **Intracellular TNF Detection Kit, 3-color combination** | | FITC, PE, PerCP-Cy5.5 | | Hu | FCM, IC | 25 tests | **340972** |
| **Anti–TNF/CD69/CD4** | 6401.1111, L78, SK3 | FITC, PE, PerCP-Cy5.5 | Ms $IgG_1$ | Hu | FCM, IC | 50 tests | **340964** |
| **TNF** | 6401.1111 | PE | Ms $IgG_1$ | Hu | IC, FCM | 50 tests | **340512** |
| **TNF** | 6401.1111 | FITC | Ms $IgG_1$ | Hu | IC, FCM | 50 tests | **340511** |
| **TNF** | 6401.1111 | APC | Ms $IgG_1$ | Hu | IC, FCM | 100 tests | **340534** |

*Recombinant Proteins*

| DESCRIPTION | FORMAT | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|
| **LT-α, Recombinant human** | | Hu | FA, ELISA Std. | 10 µg | **554619** |
| **TNF, Recombinant human** | | Hu | FA, ELISA Std. | 10 µg | **554618** |
| **TNF, Recombinant mouse** | | Ms | IC/FCM Block, FA, ELISA Std. | 10 µg | **554589** |
| **TNF, Recombinant rat** | | Rat | FA, ELISA Std. | 5 µg | **555109** |
| **Fas (Soluble) Recombinant Protein (FasDTM)** | Purified | | WB | 10 µg | **554336** |
| **Fas: Fc Chimeric Fusion Protein** | Purified | | IP | 0.1 mg | **556578** |

*BD OptEIA™ ELISA Sets and Kits*

| DESCRIPTION | APPS | SIZE | CAT. NO. |
|---|---|---|---|
| **BD OptEIA Human LT-α ELISA Set** | ELISA | Reagents for 20 plates | **550995** |
| **BD OptEIA Human TNF ELISA Kit II** | ELISA | 2 plates | **550610** |
| **BD OptEIA Human TNF ELISA Set** | ELISA | Reagents for 20 plates | **555212** |
| **BD OptEIA Human TNF Receptor I ELISA Set** | ELISA | Reagents for 20 plates | **550996** |
| **BD OptEIA Mouse TNF (Mono/Mono) ELISA Set** | ELISA | Reagents for 20 plates | **555268** |
| **BD OptEIA Mouse TNF (Mono/Poly) ELISA Kit** | ELISA | 2 plates | **559732** |
| **BD OptEIA Mouse TNF (Mono/Poly) ELISA Set** | ELISA | Reagents 20 plates | **558874** |
| **BD OptEIA Mouse TNF RII ELISA Set** | ELISA | Reagents for 20 plates | **558857** |
| **BD OptEIA Rat TNF ELISA Kit** | ELISA | 2 plates | **550734** |
| **BD OptEIA Rat TNF ELISA Set** | ELISA | Reagents for 20 plates | **558870** |
| **BD OptEIA Human TNF Receptor II ELISA Set** | ELISA | Reagents for 20 plates | **inquire** |
| **BD OptEIA Human sFasL ELISA Set** | ELISA | Reagents for 20 plates | **inquire** |

*BD™ ELISPOT Pairs, Sets and Kits*

| DESCRIPTION | APPS | SIZE | CAT. NO. |
|---|---|---|---|
| **Human Granzyme B ELISPOT Kit** | ELISPOT | 2 plates | **552573** |
| **Human Granzyme B ELISPOT Set** | ELISPOT | 10 plates | **552572** |
| **Human TNF ELISPOT Pair** | ELISPOT | 5 plates | **551882** |
| **Human TNF ELISPOT Set** | ELISPOT | 10 plates | **551446** |
| **Mouse TNF ELISPOT Set** | ELISPOT | 10 plates | **551491** |
| **Mouse TNF ELISPOT Pair** | ELISPOT | 5 plates | **551875** |

# Apoptosis

## Apoptosis Related Signal Transducers

| DESCRIPTION | CLONE | FORMAT | ISOTYPE | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|---|---|
| **A20** | E5-1619 | Purified | Ms $IgG_1$ | Hu | WB | 50 µg | **550859** |
| **β-Amyloid (a.a. 1-42)** | Polyclonal | Purified | Rab Ig | Hu | Blot, ELISA, IHC(Fr), RIA | 25 µg | **556501** |
| **β-Amyloid (a.a. 1-42) Peptide** | | | | | Block, ELISA | 250 µg | **556504** |
| **c-Myc** | 9E10 | Purified | Ms $IgG_1$ | Hu | WB | 50/150 µg | **551101/02** |
| **IkBa (pS32/pS36), Phospho-Specific** | 39A1431 | Purified | Ms $IgG_1$ | Hu | WB | 50 µg | **551818** |
| **IKKα** | Polyclonal | Purified | Rab Ig | Hu | WB | 50 µg | **550444** |
| | B78-1 | Purified | Ms $IgG_{2b}$ | Hu | IP, WB | 0.1 mg | **556532** |
| **IKKβ** | 10A9B6 | Purified | Ms $IgG_1$ | Hu, Ms, Rat | WB | 50 µg | **550621** |
| | 10AG2 | Purified | Ms $IgG_1$ | Hu | WB | 50 µg | **550743** |
| | 24 | Purified | Ms $IgG_1$ | Dog, Hu | IF, WB | 50/150 µg | **611254/55** |
| | F18-1875 | Purified | Ms $IgG_1$ | Hu | WB | 50/150 µg | **551920** |
| | Polyclonal | Purified | Rab Ig | Hu | WB | 50 µg | **550445** |
| **IKKγ (NEMO)** | 54 | Purified | Ms $IgG_{2b}$ | Dog, Hu, Rat | IF, WB | 50/150 µg | **611306/07** |
| | C73-1794 | Purified | Ms $IgG_1$ | Hu, Ms, Rat | WB | 0.1 mg | **557383** |
| | C73-429 | Purified | Ms $IgG_1$ | Hu | WB | 0.1 mg | **557384** |
| | C73-764 | Purified | Ms $IgG_1$ | Ms, Hu | IP, WB | 0.1 mg | **559675** |
| **JNK (pT183/pY185), Phospho-Specific** | 41 | Purified | Ms $IgG_1$ | Hu | WB, FCM | 50/150 µg | **612540/41** |
| **pan-JNK/SAPK1 (p49 MAPK)** | 37 | Purified | Ms $IgG_1$ | Chick, Dog, Frog, Hu, Ms, Rat | IF, IHC, WB | 50/150 µg | **610627/28** |
| **JNK1** | G151-333 | Purified | Ms $IgG_1$ | Hu | WB | 50/150 µg | **551196** |
| **JNK1/JNK2** | G151-666 | Purified | Ms $IgG_{2a}$ | Hu | IP, WB | 0.1 mg | **554285** |
| **JNKK1 (MKK4, SEK1)** | 54 | Purified | Ms $IgG_1$ | Dog, Frog, Hu, Ms, Rat | WB | 50/150 µg | **610846/47** |
| | 54 | Purified | Ms $IgG_1$ | Dros | WB | 50 µg | **612637** |
| | A32-1 | Purified | Ms $IgG_1$ | Hu | IP, WB | 0.1 mg | **556400** |
| | G282-114 | Purified | Ms $IgG_1$ | Hu | IP, WB | 0.1 mg | **554105** |
| **JNKK2** | C87-565 | Purified | Ms $IgG_{2b}$ | Hu | IP | 0.1 mg | **559676** |
| **LITAF** | 30 | Purified | Ms $IgG_1$ | Hu | IF, WB | 50/150 µg | **611614/15** |
| **MEK1** | 25 | Purified | Ms $IgG_{2a}$ | Chick, Dog, Frog, Hu, Ms, Rat | IF, IHC, IP, WB | 50/150 µg | **610121/22** |
| **MEK2** | 96 | Purified | Ms $IgG_{2a}$ | Chick, Dog, Frog, Hu, Ms, Rat | IF, IP, WB | 50/150 µg | **610235/36** |
| | A7-1 | Purified | Ms IgM | Hu, Ms | WB | 0.1 mg | **556386** |
| | Polyclonal | Serum | Rab | Hu, Ms | WB | 0.1 ml | **554098** |
| **NIK** | Polyclonal | Purified | Rab Ig | Hu | WB | 50 ug | **556569** |
| **PKR** | 23 | Purified | Ms $IgG_{2a}$ | Rat | IF, WB | 50/150 µg | **611514** |
| **PKR (p68 Kinase)** | 13 | Purified | Ms $IgG_1$ | Hu | IF, WB | 50/150 µg | **610764/65** |
| **PTEN (MMAC1, TEP-1)** | 2 | Purified | Ms $IgG_1$ | Hu, Ms, Rat | IF, WB | 50/150 µg | **611500/01** |
| | 2 | Purified | Ms $IgG_1$ | Dros | IF, WB | 50 µg | **612649** |
| | A2B1 | Purified | Ms $IgG_1$ | Hu | WB | 100 µg | **559600** |

## BD PowerBlot[SM] Protein Array Screening Service

BD PowerBlot[SM] Protein Array Screening Service is a patented, rapid screening service that simultaneously measures changes of expression and phosphorylation levels of 40 to 1000 of proteins. Small-scale screening service is available for 40 antibodies or 150 antibodies of your choice. Alternatively, select functional screens, each involving 80 – 300 antibodies, such as the Apoptosis Array, Cell Cycle Array, or the Phospho-Specific Array.

This assay uses an extensive library of quality antibodies. Levels of protein expression of fully characterized proteins are measured and stored for analysis. The multiple antibody approach will generate systematic data regarding the levels, dynamics, and distribution of signal transduction proteins under both normal and disease conditions.

With each service performed, we provide you with raw data, comparative analyses, and a summary of your results.
For more information please visit **www.powerblot.com.**

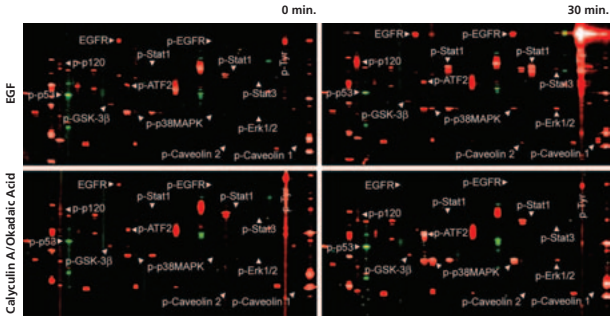
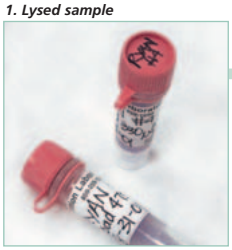


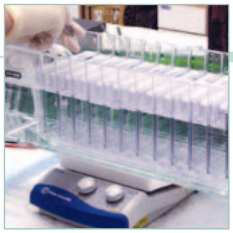

*Figure 23. The BD PowerBlot[SM] Phospho Specific Array measures the phosphorylation states of more than 30 signaling proteins, plus total protein expression for each of the phospho-specificities. This screen targets several signaling pathways and can be used to examine intracellular perturbations more subtle than the up- or down-regulation of protein expression. Shown below are data from our Phospho-Specific Array, using A431 cells stimulated with EGF or with calyculin A and okadaic acid. With each service performed, we provide you with the raw data, comparative analyses, and a summary of your results*

***1. Lysed sample***



*Send us your control and treated samples.*

***2. SDS-PAGE***

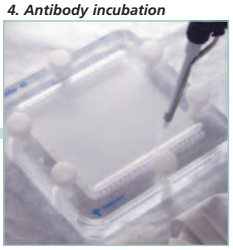


*Submitted samples are loaded for gel electrophoresis.*

***3. Transfer***

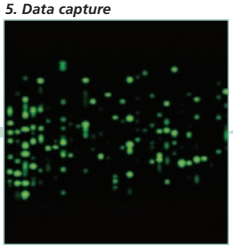
*Proteins are transferred to PVDF membranes.*

***4. Antibody incubation***



*Membranes containing samples are probed with 40 – 1000 mAbs (depending on the service).*

***5. Data capture***



*Electronic images of blots are captured using the Odyssey™ Infrared Imaging System. Fluorescent detection enables a significantly broader linear array range compared to chemi-luminescence detection.*

***6. Data analysis***

*Images are subjected to automatic spot finding and spot matching using PDQuest software (Bio-Rad).*

***7. Data summary***

| BD PowerBlot | Templat A | | | Wilson | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Samples CON,STAURO | | | | | | | | | | | |
| Gel Name | Lane | Predicted Mol. Wt. | Observed Mol. Wt. | Protein ID | Quantity | Normalized Quantity | Change Run2 +/- | Fold Change Run1 | Change Run2 +/- | Fold Change Run2 | Change Run3 +/- | Fold Change Run3 |
| CONAR1 | 2 | 182 | 198 | AF6 | 5573 | 4928 | - | 10.63 | - | 62.68 | - | 6.52 |
| CONAR2 | 2 | 182 | 198 | AF6 | 36989 | 21118 | | | | | | |
| CONAR3 | 2 | 182 | 198 | AF6 | 4744 | 2195 | | | | | | |
| STAUROAR1 | 2 | 182 | 198 | AF6 | 462 | 464 | | | | | | |
| STAUROAR2 | 2 | 182 | 198 | AF6 | 1177 | 337 | | | | | | |
| STAUROAR2 | 2 | 182 | 198 | AF6 | 1177 | 337 | | | | | | |
| CONAR1 | 6 | 26 | 29 | Bcl-2 | 13369 | 11821 | + | 1.91 | | 1.01 | - | 1.71 |
| CONAR2 | 6 | 26 | 29 | Bcl-2 | 21159 | 12080 | | | | | | |
| CONAR3 | 6 | 26 | 29 | Bcl-2 | 44093 | 20406 | | | | | | |
| STAUROAR1 | 6 | 26 | 29 | Bcl-2 | 22475 | 22532 | | | | | | |
| STAUROAR2 | 6 | 26 | 29 | Bcl-2 | 41736 | 11941 | | | | | | |
| STAUROAR2 | 6 | 26 | 29 | Bcl-2 | 41736 | 11941 | | | | | | |
| CONAR1 | 5 | 125 | 146 | BM28 | 28825 | 25488 | + | 2.16 | + | 4.84 | + | 6.58 |
| CONAR2 | 5 | 125 | 146 | BM28 | 72911 | 41627 | | | | | | |
| CONAR3 | 5 | 125 | 146 | BM28 | 66235 | 30653 | | | | | | |
| STAUROAR1 | 5 | 125 | 146 | BM28 | 54881 | 55020 | | | | | | |
| STAUROAR2 | 5 | 125 | 146 | BM28 | 704772 | 201649 | | | | | | |

*Data is exported to an electronic spreadsheet that indicates raw signal intensity data, normalized data, and comparative analysis.*

***8. Bioinformatics***

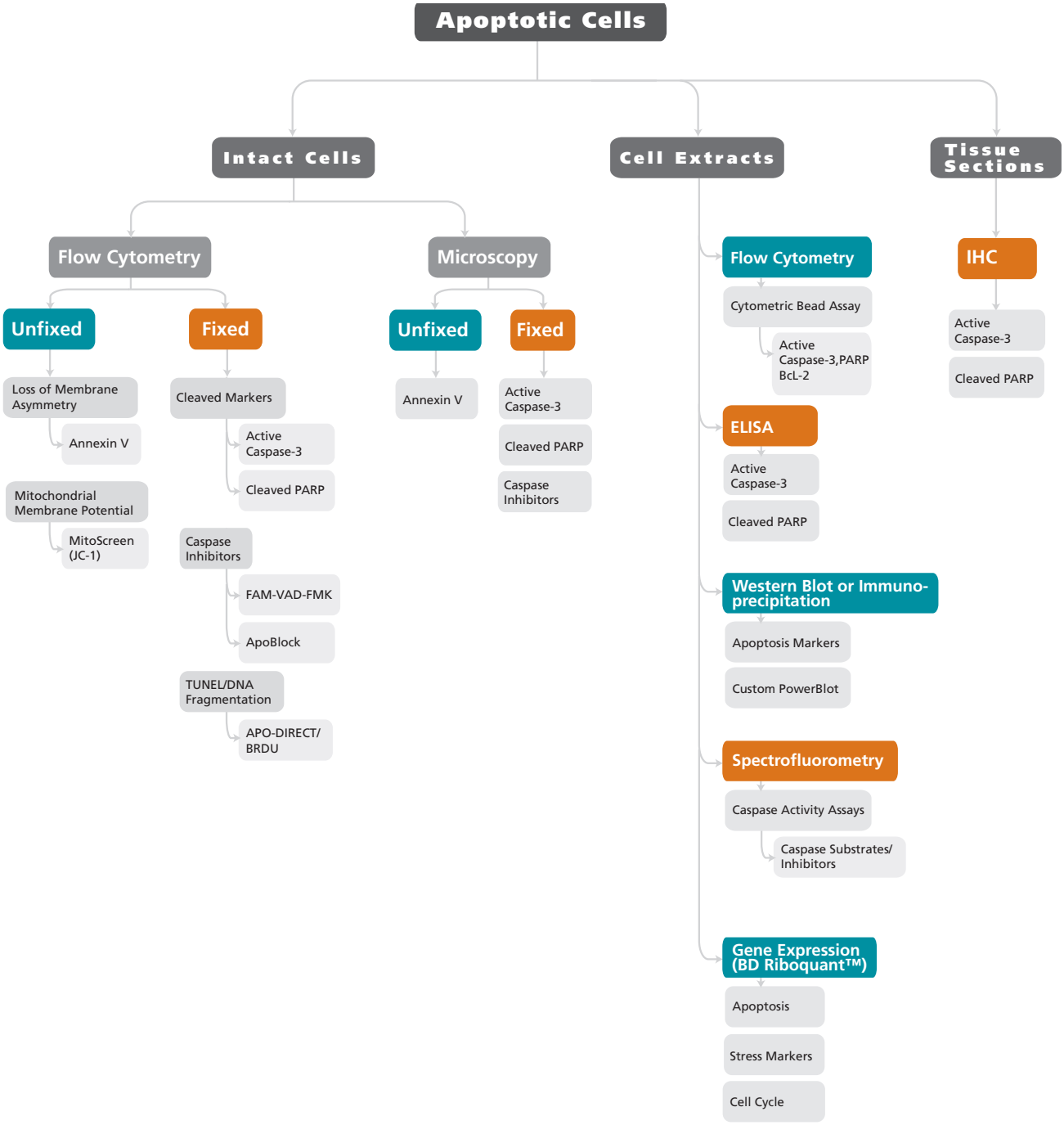
A summary file (see below) is provided which lists all protein expression changes detected, in order of confidence, 1 through 10, with 10 being the highest confidence. The confidence level is based on fold change, reproducibility, and signal intensity.

# Apoptosis

## Summary. Application Decision Tree

In summary, although the underlying cellular mechanisms of the assays discussed here may vary, each assay is uniquely valuable for identifying cells undergoing apoptosis. Apoptosis is a highly conserved process; therefore, the majority of apoptosis assays can be used for a wide variety of model systems. It is nevertheless important to note that the optimal method for identification of apoptotic events will depend on the model system used, on the cell or tissue type, as well as on the mode of induction of apoptosis. The decision to select a particular assay(s) often relies on a combination of the sample material available, the technique desired, and the cellular phenomena under examination. The decision tree below shows a breakdown according to the type of sample and the method used, and is intended to help researchers choose the most suitable methods for their particular experiment.

### Apoptosis Decision Tree



## Introduction

Somatic cells proliferate to support tissue and organismal growth and to replace damaged cells. Proliferation is also a fundamental response that underlies cellular mechanisms involved in immunity, inflammation, hematopoiesis, neoplasia, and other biological responses. (For example, in adaptive immunity, specific T and B lymphocytes undergo clonal expansion (division and differentiation) in response to foreign antigenic stimulation. Cell growth, replication, and division in eukaryotic cells occur according to a highly controlled series of events called the cell cycle.[1]

### The Cell Cycle

The cell cycle can be subdivided into two major stages: interphase (a phase between mitotic events) and mitosis (***Figure 25***). There are three distinct, successive stages within interphase, called G1, S, and G2 phases. During G1, cells "monitor" their environment and upon receipt of requisite signals, induce growth (synthesize RNA and proteins). If conditions are right, cells "commit" to DNA synthesis (S phase) and replicate their chromosomal DNA. A G2 phase follows in which cells continue to grow and prepare for mitosis.

G2 allows time for the cell to ensure DNA replication is complete before initiating mitosis. In mitosis (division), there are four successive phases called prophase, metaphase, anaphase, and telophase that are accompanied by cytoplasmic division (cytokinesis) giving rise to two daughter cells. For the most part, upon completion of the process, each daughter cell contains the same genetic material as the original parent cell.

In addition to these specific stages, the G0 phase has been described for cells that exit from the cell cycle and enter a quiescent, non-dividing state. In response to external stimuli, some quiescent cells may undergo reactivation and express early response genes. The G0–G1 transition is marked by cell growth with measurable increases in newly-synthesized RNAs and proteins. This transition is reflected by the increased forward-scattered light signals (blast transformation) and by the expression of early cell-surface activation antigens (eg, CD69 and IL-2Rα/CD25) on cells as detected by immunofluorescent staining and flow cytometric analysis of lymphocytes.

Determination of the frequency and the nature of cells that synthesize DNA and progress through the cell cycle is crucial for better understanding of the cellular basis of such responses in health and disease. Multiple techniques are available to measure proliferative responses by cells. Techniques utilizing immunofluorescence, flow cytometry, and immunohistochemistry have grown in importance because of their capacity to determine the cell cycle status or tissue localization of individual cells within proliferating populations.

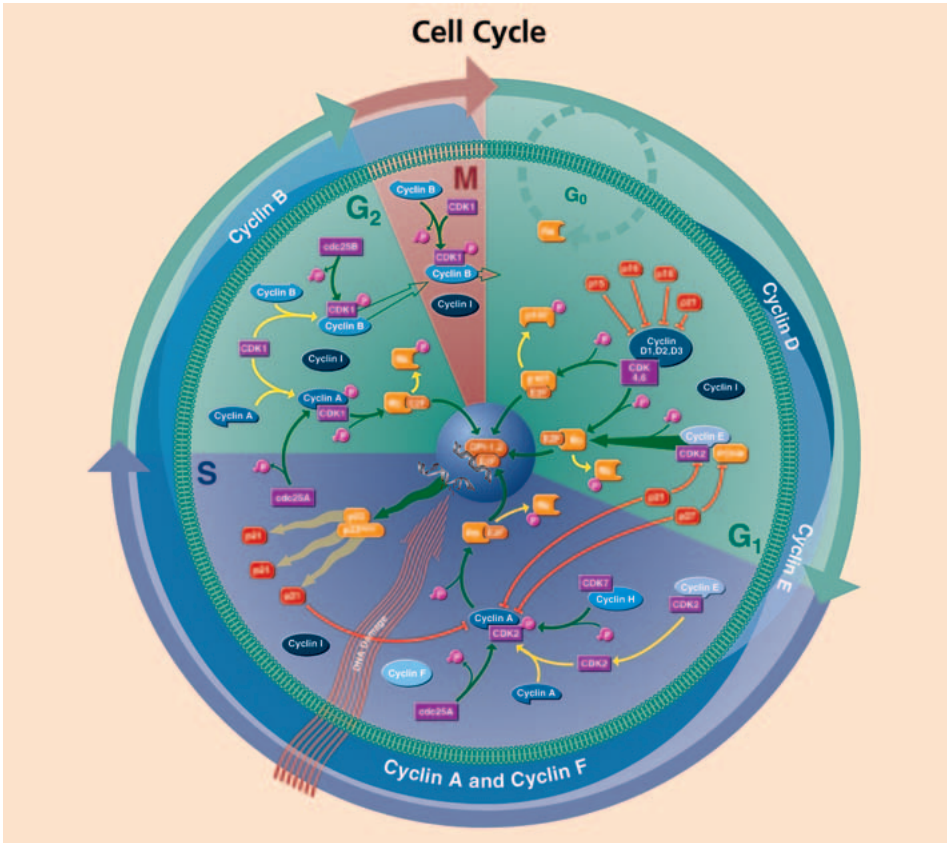



*Figure 24. Cell Cycle overview*

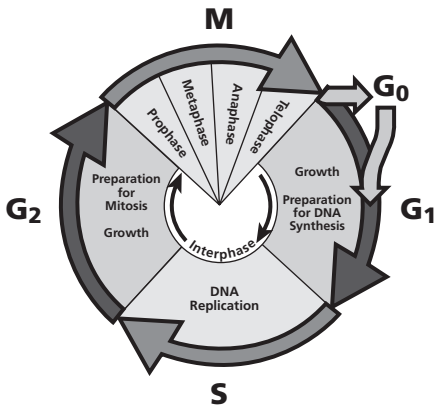



*Figure 25. Cell Cycle phases*

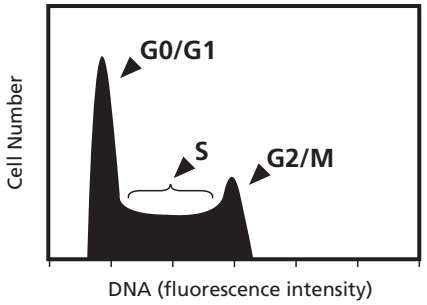



*Figure 26. Analysis of cellular DNA content.*

## Analysis of Cellular DNA Content

- Flow cytometric analysis of cell cycle
- Compatible with multicolor analysis of other antigens
- Amenable to 96-well plate analysis

A number of fluorescent probes have been developed for the flow cytometric analysis of cycling cells.[1] The prototype for single-color flow cytometric analysis of cycling cells uses a fluorescent nucleic acid dye, such as propidium iodide (PI), to stain the total cellular DNA content. This sort of staining generates characteristic cellular DNA content profiles as shown in ***Figure 27.*** These histograms can be separated into regions that represent cells within G0/G1, S, and G2/M phases of the cell cycle. Cells in the G0/G1 phase (before DNA synthesis) have a defined amount (1×) of DNA (ie, a diploid chromosomal DNA content). During S phase (DNA synthesis), cells contain between 1× and 2× DNA levels. Within the G2 or M phases (G2/M), cells have a 2× amount of DNA (ie, a tetraploid chromosomal DNA content). In this way, it is possible to identify the proportion of cells in each of the three interphase stages of the cell cycle.

A protocol for staining DNA for cell cycle analysis with propidium iodide can be found in the technical data sheet for related products, in chapter 9 of *Techniques for Immune Function Analysis: Application Handbook 1st Edition* or on our web site: **www.bdbiosciences.com/pdfs/manuals/02-8100055-21-A1.pdf**

Cell cycle analysis with propidium iodide can be done using the BD FACSArray™ bioanalyzer, in a 96-well format. A detailed protocol is available in the Application Notes section if our web site: **www.bdbiosciences.com/literature** (download page)

**bdbiosciences.com/pdfs/whitePapers/04-7900030-20-A1.pdf**

### Propidium Iodide

Propidium iodide (PI) is the most widely used fluorescent dye for staining DNA in whole cells or isolated nuclei.[2,3] PI intercalates into the DNA helix of fixed and permeabilized cells. Because PI can stain both double-stranded RNA (dsRNA) and DNA (dsDNA), cells must be treated with RNase to ensure that PI staining is DNA specific. BD Biosciences – Pharmingen offers PI/RNAse staining buffer suited for this purpose (Cat. No. 550825). PI does not cross the intact plasma membrane of viable cells. However, PI can readily enter dead cells, cells in late stages of apoptosis, or fixed cells, that have damaged plasma membranes. For this reason, PI is also widely used as a discriminator of live and dead cells in experiments using immunofluorescent staining of unfixed cells with flow cytometric analyses. For this purpose, BD Biosciences – Pharmingen offers a Propidium Iodide Staining Solution (Cat. No. 556463).

### 7-aminoactinomycin D (7-AAD)

7-AAD is a DNA-specific dye that can be used for staining fixed and permeabilized cells to determine the DNA content profiles of cell populations in multicolor flow cytometric analyses.[2,4]

7-AAD can be used in the multicolor analysis of cellular DNA content (cell cycle status), and two or more florescent label antibodies and with BrdU staining.

7-AAD will not enter live cells but will readily stain dead cells. For this reason, 7-AAD is also used as a live–dead cell discriminator for flow cytometric analyses. A solution of 7-AAD for viability staining is available from BD Biosciences – Pharmingen as BD Via-Probe™ (Cat. No. 555815).

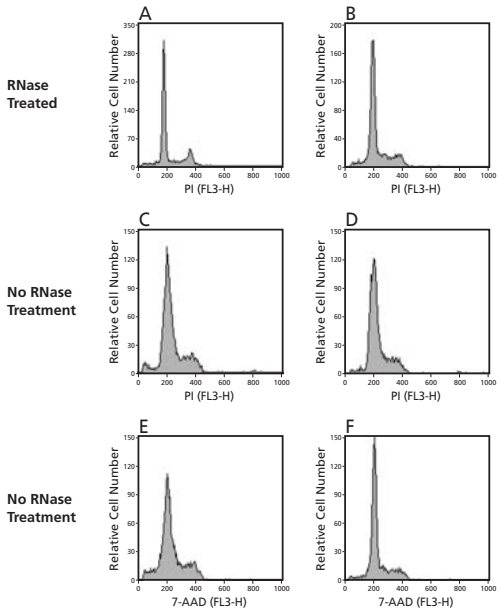



***Figure 27. DNA content histograms:*** *PI versus 7-AAD. Cells from two mouse T cell lines, MGG3 (panels A, C, and G) and C20.4 (panels B, D, and F), were harvested, washed 1× with staining buffer, and fixed with ice-cold 70% ethanol (4°C, 1 hr). Cells were then washed to remove ethanol. Panels A and B: Cells were treated with 100 µg of RNase A (30 min, 37°C) and were stained with 10 µg/ml of PI. Panels C and D: Cells were not treated with RNase A before PI staining. Panels E and F: Cells were stained with 10 µg/ml 7-AAD. Notice that PI staining with RNase treatment (Panels A and B) gives a very clean DNA profile compared to no RNase treatment (Panels C and D). The DNA profiles obtained by 7-AAD staining of these same cells can be more variable with either higher (Panel E) or similar (Panel F) CV's when compared with the corresponding patterns for RNase-treated, PI-stained cells. Because of this variability, 7-AAD is not recommended as the first choice for single-color DNA content analysis*

| DESCRIPTION | FORMAT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|
| **Propidium Iodide Staining Solution** | Solution | FCM | 2 ml | **556463** |
| **7-AAD Staining Solution** | Solution | FCM | 2 ml | **559925** |
| **PI/RNase Staining Buffer** | Solution | FCM | 100 ml | **550825** |

PI is excited by the 488 nm wavelength of light with broad emission centered around 617 nm.

7-AAD is excited by the 488 nm/ 530 nm wavelength of light with emission peak around 650 nm

### References

1. Cell organization, subcellular structure, and cell division. 1995. In *Molecular Cell Biology. Third Edition*. H. Lodish, D. Baltimore, A. Berk, S. L. Zipursky, P. Matsudaira, and J. Darnell, eds. W. H. Freeman and Company, New York, pp. 141–188.
2. Crissman, H. A., and J. A. Steinkamp. 1987. Multivariate cell analysis. Techniques for correlated measurements of DNA and other cellular constituents. In *Techniques in Cell Cycle Analysis.* J. W. Gray, and Z. Darzynkiewicz, eds. Humana Press, Clifton, New Jersey, 163–206.
3. Noguchi, P. 1991. Use of flow cytometry for DNA analysis. In *Current Protocols in Immunology*. J. Coligan, A. Kruisbeek, D. Margulies, E. Shevach, and W. Strober, eds. Green Publishing Associates and Wiley-Interscience, New York. Section 5.7.1–5.7.4
4. Rabinovich, P. R. Torres, and D. Engel. 1986. Simultaneous cell cycles analysis and two-color surface immunofluorescence using 7-amino-actinomycin D and single laser excitation:Applications

## BD CycleTEST™ PLUS DNA Reagent Kit

The BD CycleTEST™ PLUS DNA Reagent Kit provides a set of reagents for isolating and staining cell nuclei from fresh or frozen solid tissue specimens, or cell suspensions. Flow cytometric analysis of differentially stained normal and tumor cells is used for the identification of abnormal DNA stemlines and to estimate the DNA index (DI) and cell cycle phase distributions of these stemlines.

Uniform suspensions of single nuclei are prepared for DNA staining and flow cytometric analysis from surplus solid tissue specimens or cell suspensions. The BD CycleTEST PLUS assay involves dissolving the cell membrane lipids with a nonionic detergent, eliminating the cell cytoskeleton and nuclear proteins with trypsin, digesting the cellular RNA with an enzyme, and stabilizing the nuclear chromatin with spermine. Flow cytometric DNA stemlines analysis is being used for characterizing clonal diversity and researching the potential for clinical progression of these human neoplasms.

For detailed information regarding the kit and procedures please view the *technical data sheet* (Cat. No. 340242) online at **www.bdbiosciences.com**.

| DESCRIPTION | FORMAT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|
| **BD CycleTEST PLUS DNA Reagent Kit** | | FCM | 40 tests | **340242** |

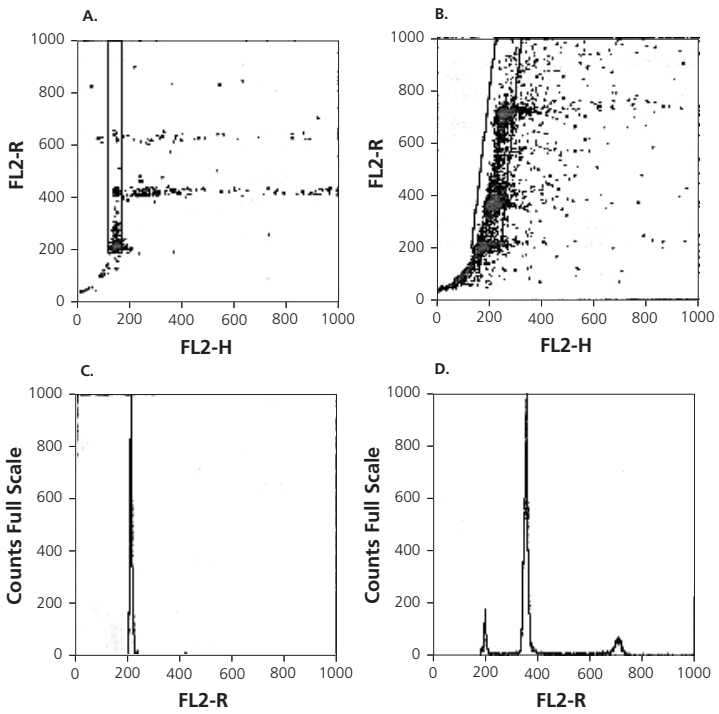



***Figure 28.*** *(A) Peripheral blood mononuclear cells (PBMCs): FL2- Width versus FL2-Area dot plot showing a singlet gate, which excludes aggregates. PBMCs may be mixed with tumor samples as an internal control. (B) Breast tumor tissue: FL2-Width versus FL2-Area dot plot showing a singlet gate, which excludes aggregates. (C) FL2-A DNA histogram of PBMCs, which has been gated to exclude aggregates. (D) FL2-A DNA histogram of breast tumor tissue, prepared by in vitro FNA, which has been gated to exclude aggregates.*

## Cell Proliferation Assays

### Determination of S Phase Activity using Bromodeoxy-Uridine

A method to measure *de novo* DNA synthesis is the analysis of cellular incorporation of bromodeoxy-uridine (BrdU), an analog of the DNA precursor thymidine, during S (DNA synthesis) phase. The incorporated BrdU can be readily detected by anti-BrdU-specific antibodies (that do not recognize thymidine). The BrdU-positive cells can then be detected by flow cytometry or immunohistochemistry.

BD Biosciences – Pharmingen offers BrdU detection kits for flow cytometry and immunohistochemistry. These kits do not employ harsh DNA denaturing agents in the cell preparation and staining protocols, making them compatible with multicolor fluorescent and/or immunohistochemical staining.

### BrdU Flow Kits

- Flow cytometric analysis of cell proliferation and cell cycle
- Compatible with multicolor analysis of other antigens
- Amenable to 96-well plate analysis

Flow cytometric analysis of immunofluorescent staining of incorporated bromodeoxyuridine (BrdU) enables determination of the frequency and nature of individual cells that have synthesized DNA in the course of a specific time interval. The incorporated BrdU can be detected with specific fluorescence-labeled anti-BrdU antibodies. The BrdU staining can be combined with a dye that binds to total DNA, such as 7-AAD. With this combination, two-color flow cytometric analysis enables characterization of cells that have actively synthesized DNA (BrdU incorporation) with their cell cycle position (ie, G0/1, S, or G2/M phases as defined by 7-AAD staining intensities).

Prolonged exposure of cells to BrdU allows for the identification and analysis of actively-cycling, as opposed to non-cycling, cell fractions. Pulse-labeling of cells with BrdU at various time points permits a detailed examination of cell cycle kinetics in *in vitro* and *in vivo* labeling systems.

Importantly, the BD Pharmingen™ BrdU Flow Kit is that it provides reagents for immunofluorescent BrdU staining with a protocol that is compatible with the use of additional fluorescent antibodies specific for other cellular molecules. Cell surface antigens or intracellular proteins (eg, cytokines or cyclins) whose expression or activity may be related to the cell's activation, entry and progression through cell cycle, or cell death can be detected together with BrdU staining. This is possible, because the BrdU Flow Kit staining protocol avoids DNA-denaturing agents such as acid, ethanol, and high temperatures that can change cellular light-scattering characteristics and limit the recognition of antigens by fluorescent antibodies.[1-3] The kit ensures consistent results by providing detailed instructions and all critical reagents necessary to implement the staining protocol.

Detailed protocols for BrdU incorporation and staining can be found in the respective user manuals, available at **www.bdbiosciences.com/literature** (download page).
**www.bdbiosciences.com/pdfs/manuals/02-8100055-21-A1.pdf**

To support high throughput analysis of proliferating cells, we have developed a BrdU staining protocol utilizing 96-well, U-bottom plates, which can be used on BD flow cytometers equipped with a plate loader, or using the new BD FACSArray™ bioanalyzer. For more details, refer to the article in ***HotLines v. 8, No. 3 (Winter 20004)*** (available on the literature download page).
**www.bdbiosciences.com/pdfs/newsletters/03-7900030-36-A1.pdf**

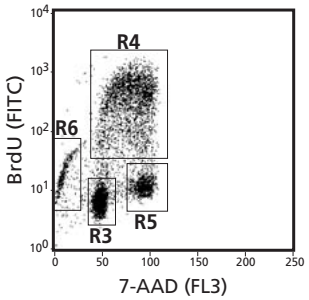



***Figure 29. BrdU and 7-AAD coexpression profile for an actively-proliferating cell population.*** *D10.G4.1 T cells were pulsed with 10 µM BrdU for 30 minutes. The cells were then stained for BrdU and 7-AAD using the BrdU Flow Kit and analyzed by flow cytometry. As shown by the boxed region gates, significant proportions of cells are found to occupy distinct cell cycle phases including G0/G1, S, and G2/M. Region 6 identifies apoptotic cells as determined by their sub-G0/G1 levels of DNA (stained by 7-AAD), Region 3 shows cells within the G0/G1 phases (39%) of the cycle, whereas Region 4 includes BrdU⁺ or S phase cells (39%), with Region 5 showing cells that occupy the G2/M phases (14%).*

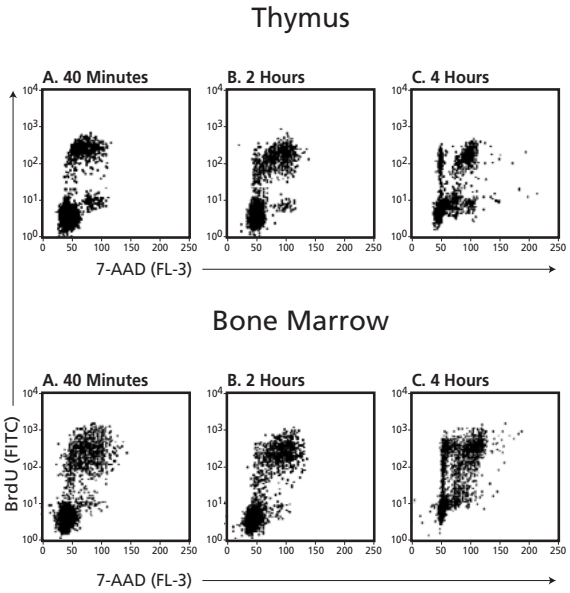



***Figure 30. Detection of in vivo-cycling cells.*** *C57BL/6 mice were injected i.p. with 1 mg BrdU in solution for various time intervals. Animals were sacrificed at 40 minutes, 2 hours, and 4 hours post injection. Thymus and bone marrow cell suspensions were then prepared and stained for incorporated BrdU (FITC-anti-BrdU) and total DNA (7-AAD) levels. The 40–minute time point shows the characteristic "horseshoe" pattern that is seen for cell populations that are pulsed with BrdU for a short time. Cells from mice that were pulsed for 2 hours also show the horseshoe pattern. However, another cell population of G0/G1 cells that has incorporated BrdU and has returned to the G0/G1 phase is now detectable. These cells are positive for BrdU but have 1× DNA levels (ie, diploid chromosomal DNA levels) as determined by their cellular DNA content (7-AAD level). The 4–hour time point has an even larger population of BrdU⁺ G0/G1 phase cells.*

More examples of multi parameter BrdU analyses incorporating RNA dyes and staining for cell surface phenotype to determine the S phase activity of particular subpopulations can be found in Chapter 9 of ***Techniques for Immune Function Analysis: Application Handbook 1st Edition*** or on our web site:
**www.bdbiosciences.com/pdfs/manuals/02-8100055-21-A1.pdf**

## Phosphorylation of Serine 10 in Histone H3

Cells protect DNA by organizing it as a higher-order nucleoprotein complex termed chromatin. Eukaryotic cells have a mechanism for condensing and decondensing chromatin.[1] Chromatin condensation is particularly evident during mitosis and cell death induced by apoptosis. Chromatin decondensation is necessary for DNA replication, repair, recombination, and transcription. Histones are among the numerous DNA-binding proteins that control the level of DNA condensation. Post-translational modification of histone tails plays a critical role in the dynamic condensation/decondensation that occurs during the cell cycle. Phosphorylation of the serine 10 residue of the N-terminal tail of histone H3 is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis.[2]

Histone H3 phosphorylation at Ser 10 begins during prophase, with peak levels detected during metaphase, ultimately followed by a general decrease in the amount of phosphorylation during the progression through the cell cycle to telophase. A similar correlation can be observed during meiosis.[3] In addition, activation of the MAP kinase cascade by growth factors, stimuli, stress, or UV irradiation leads to phosphorylation of H3 (Ser10) and results in the transcription of different genes, such as c-fos and c-jun.

BD Biosciences – Pharmingen introduces AlexaFluor® 647 anti-Human Histone H3 (Ser10) (clone HTA28). This antibody is suitable for flow cytometry in conjunction with a DNA dye, such as PI.

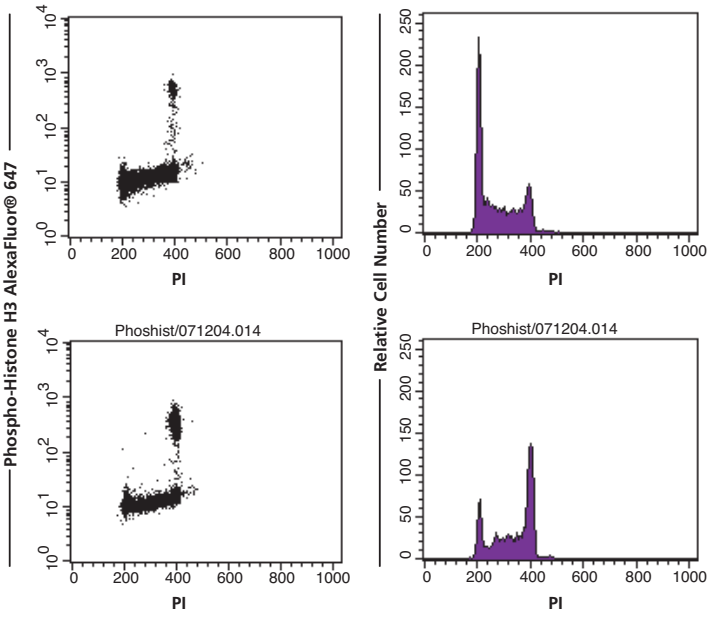



*Figure 31. C20.4 cells were either untreated (upper panels) or treated (lower panels) with100 ng/ml of Colcemid (G2+M cell cycle inhibitor). Cells were subsequently fixed, permeabilized and stained with AlexaFluorR 647 – Phospho-Histone H3 versus PI.*

### References


1. Wolffe, A. (1998) Chromatin: Structure and Function, Academic Press
2. Gurley, L.R. *et al*. (1978) Histone phosphorylation and chromatin structure during mitosis in Chinese hamster cells. *Eur. J. Biochem*. 84, 1–15)
3. Wei, Y. *et al*. (1999) Phosphorylation of histone H3 is required for proper chromosome condensation and segregation. *Cell* 97, 99–109
4. 3. Mahadevan, L.C. *et al*. (1991) Rapid histone H3 phosphorylation in response to growth factors, phorbol esters, okadaic acid, and protein synthesis inhibitors. *Cell* 65, 775–783


## BrdU Flow Analysis

| DESCRIPTION | CLONE | FORMAT | ISOTYPE | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|---|---|
| **BrdU** | 3D4 | Purified | Ms $IgG_1$, κ | Hu | IHC(Fr), IC/FCM | 0.1 mg | **555627** |
| **BrdU** | 3D4 | FITC Set | Ms $IgG_1$, κ | Hu | FCM, IC/FCM | 100 tests | **556028** |
| **BrdU** | 3D4 | PE Set | Ms $IgG_1$, κ | Hu | FCM, IC/FCM | 100 tests | **556029** |
| **FITC BrdU Flow Kit** | | FITC Kit | | | IC/FCM | 50 tests | **559619** |
| | | | | | IC/FCM | 4 × 50 tests | **557891** |
| **APC BrdU Flow Kit** | | PE Kit | | | IC/FCM | 50 tests | **552598** |
| | | | | | IC/FCM | 4 × 50 tests | **557892** |
| **BrdU solution** | | | | | | 5.0 ml | **550891** |

## BD FastImmune™ Proliferation Analysis in Whole Blood

| DESCRIPTION | CLONE | FORMAT | ISOTYPE | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|---|---|
| **BrdU** | B44 | Purified | Ms $IgG_1$ | | FCM | 100 tests | **347580** |
| **BrdU** | B44 | FITC | Ms $IgG_1$ | | FCM | 100 tests | **347583** |
| **BD Fastimmune Anti-BrdU** | B44 | FITC | Ms $IgG_1$ | Hu | Prolif, FCM, IC | 50 tests | **340649** |

## Proliferation of Histone H3

| DESCRIPTION | CLONE | FORMAT | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|---|
| **Phosph-Histone H3 (S10)** | HTA28 | AlexaFluor® 647 | Hu | FCM | 50 tests | inquire |

## Cell Proliferation Assays *(continued)*

### BrdU *In-Situ* Detection Kit

- Immunohistochemical analysis of cell proliferation in all species and fixation methods
- Preserves tissue morphology
- Compatible with immunohistochemical staining of other antigens

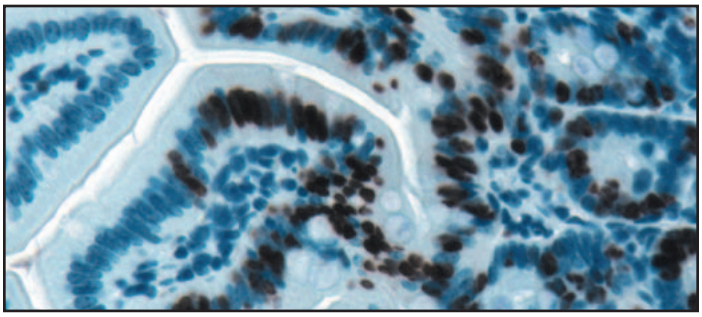
The BD Pharmingen™ BrdU *In-Situ* Detection Kit is designed for immunohistochemical staining of incorporated BrdU in frozen sections, formalin-fixed paraffin-embedded sections, and cultured or isolated cells on slides in conjunction with staining of other antigens. The monoclonal antibody against BrdU works in all species tested (human, mouse, and rat) and provides improved specific staining with minimal background. The directly biotinylated mouse antibody eliminates the need for a species-specific secondary antibody thus allowing the *in situ* kit to be used in mouse tissues.

The BrdU *In-Situ* Detection Kit features BD™ Retrievagen A, an antigen retrieval solution designed specifically to unmask antigenic sites, preserve tissue morphology, and enable simultaneous staining of other surface antigens in conjunction with BrdU. This important feature of the kit enables study of the proliferation state of phenotypically defined cells within the micro-environment of tissues. Consistent results are assured by providing all critical reagents in addition to a comprehensive series of protocols for *in vivo* and *in vitro* studies. Control slides are provided in the kit to serve as a reference.

**References**


1. Toba, K., E.F. Winton and R. A. Bray. 1992. Improved staining method for the simultaneous flow cytofluormetric analysis of DNA content, S phase fraction, and surface phenotype using single laser instrumentation. *Cytometry* 13:60.
2.. Sasaki, K. , S. Adachi, T. Yamamoto, T. Murakami, K. Tanaka and M. Takahashi. 1988. Effects of denaturation with HCl on the immunological staining of bromodeoxyuridine incorprated into DNA. *Cytometry* 9:93.
3. Houck, D.W. and M. R. Loken. 1985. Simultaneous analysis of cell surface antigens, bromodeoxyuridine incorporation and DNA content. *Cytometry* 6: 531.
5. Cell cycle Analysis by Antobodies for Flow Cytometry, Western Blot and IHC Analysis of Cyclins, Cyclin Dependent Kinases and Cell Cycle Regulators


**A.**



**B.**

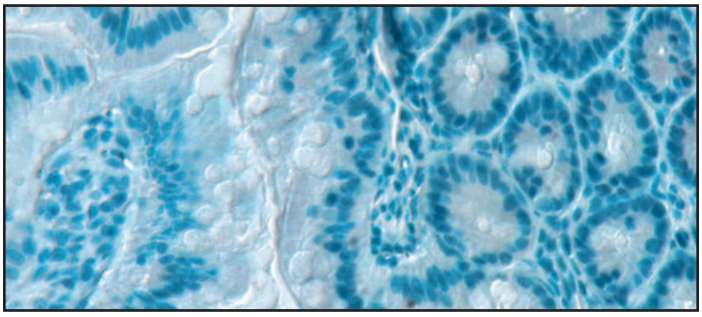


***Figure 32. Immunohistochemical staining of BrdU in paraffin sections.*** *BALB/c mice were injected with 1 mg BrdU via the intra-peritoneal route. After 24 hrs, the spleen, thymus, and gastro-intestinal tract were harvested and processed for paraffin sections. Mice injected with PBS served as the negative control. Immunohistochemical staining of BrdU was performed using the BrdU In-Situ Detection Kit on paraffin sections of the mouse gastro-intestinal tract. Proliferating cells in the crypts that incorporated BrdU can be identified by the dark brown color in their cell nuclei (A) in contrast to the control (B). Magnification 400×.*

| DESCRIPTION | APPS | SIZE | CAT. NO. |
|---|---|---|---|
| **BrdU *In-Situ* Detection Kit** | IHC(Fr), IHC(F), IHC(Zn) | 50 tests | **550803** |
| **BrdU *In-Situ* Detection Kit II** | IHC(Zn), IHC(F), IHC(Fr) | 200 tests | **551321** |

## Analysis of Cyclins, Cyclin Dependent Kinases, and Cell Cycle Regulators

An understanding of cell cycle progression is essential for comprehension of disease pathology and for the rational design of novel drugs to adjust incorrectly regulated cell proliferation. Accelerated cell division can result in cancers that proliferate rapidly unless anti-cancer agents intervene. External stimuli can induce halt of cell cycle, quiescent state, which can eventually lead to cell death.

Progression of mammalian cell cycle is regulated by phosphorylation of many key proteins. Several classes of cyclins (A-E) interact with, and act as regulatory subunits for cyclin-dependant kinases (cdks).

Cyclins and cyclin-dependent kinases (cdks) are essential for cell cycle control in eukaryotes. Cyclins (regulatory subunits) bind to cdks (catalytic subunits) to form active cyclin-cdk complexes. Cdk subunits by themselves are inactive and binding to a cyclin is required for their activity. Active cyclin-cdk complexes drive cells through particular cell cycle phases, called *checkpoints*, by phosphorylating the unique sets of protein substrates essential for cells to achieve transition to the next phase.

Cyclins A, B1, D, and E undergo periodic synthesis and degradation, thereby providing a mechanism to regulate cdk activity throughout the cell cycle. Cdk activity is further regulated by activating or inhibitory phosphorylation, and by small proteins (p15, p18, p19, p21, and p27), called inhibitors of cdk activity, that bind to cyclins, cdks, or cyclin-cdk complexes. BD Biosciences – Pharmingen offers an expanding line of antibodies and reagents for the analysis of cyclins, cdks, and cell cycle regulators.

Detailed protocols for the analysis of cyclins by flow cytometry are available on our web site: **www.bdbiosciences.com/pharmingen/protocols/Human_Cyclins.shtml**

Retinoblastoma protein (Rb) also plays a key role in the control of cell division and differentiation. Rb is a nuclear protein that undergoes cell cycle dependent phosphorylation. Underphosphorylated forms predominate in G0 and early G1, whereas more phosphorylated forms are present in S, G2 and M.

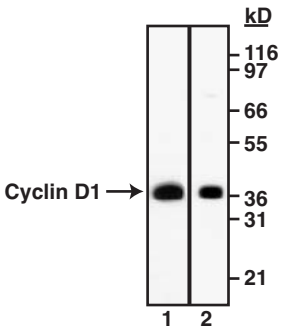



***Figure 33. Western blot analysis of Cyclin D1.*** *Lane 1, WI-38 human diploid fibroblast cell lysate; lane 2, LNCaP, a human prostate adenocarcinoma cell line. Anti-cyclin D1 (Cat. No. 554180) identifies cyclin D1 as an ~36 kD band in both cell types.*

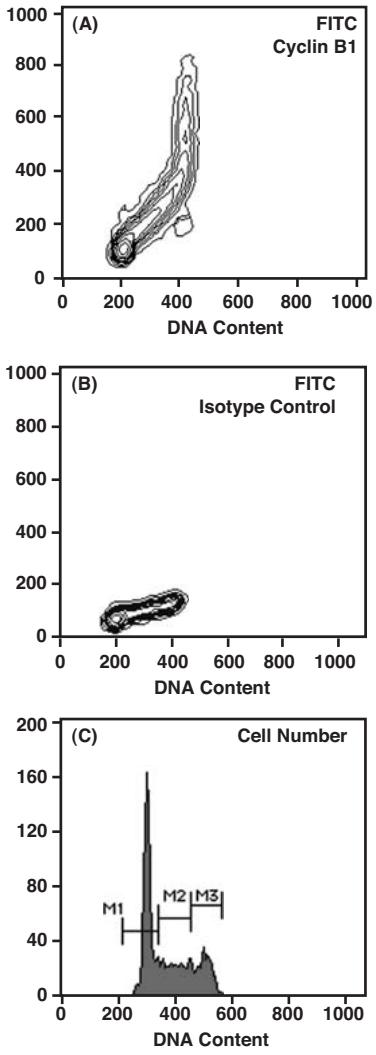



***Figure 34. Cell cycle expression of cyclin B1.*** *Proliferating MOLT-4 human leukemia cells were stained with (A), FITC-conjugated cyclin B1, or (B) a FITC-conjugated isotype control. (Cat. No. 554108). DNA was stained with propidium iodide. Cyclin B1 expression was low in G0/G1, increased in S and was maximal in G2/M. (C) Marker 1 (M1): G0/G1. Marker 2 (M2): S. Marker 3 (M3): G2/M.*

# Cell Cycle and Proliferation

## Cyclins and CDKs

| DESCRIPTION | CLONE | FORMAT | ISOTYPE | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|---|---|
| **Cdc25B** | 23 | Purified | Ms $IgG_1$ | Chick, Dog, Hu, Ms, Ra | IF, IHC, IP, WB | 50/150 µg | **610527/28** |
| **Cdc25C** | C2-2 | Purified | Ms $IgG_1$ | Hu | WB | 0.1 mg | **556576** |
| | TC113 | Purified | Ms $IgG_1$ | Hu | IF, IP, WB | 50 µg | **550920** |
| | TC14 | Purified | Ms $IgG_1$ | Hu | IP, WB | 50 µg | **550922** |
| **Cdc27** | 35 | Purified | Ms $IgG_{2b}$ | Dog, Hu, Ms, Rat | IF, IP, WB | 50/150 µg | **610454/55** |
| **Cdc34** | 17 | Purified | Ms $IgG_{2a}$ | Chick, Dog, Hu, Ms, Rat | IF, WB | 50/150 µg | **610249/50** |
| | Polyclonal | Serum | Rab | Hu | WB | 0.1 ml | **559679** |
| **Cdc37** | Polyclonal | Serum | Rab Ig | Hu | WB | 0.1 ml | **558895** |
| | 15 | Purified | Ms $IgG_1$ | Dog, Hu, Ms, Rat | IF, WB | 50/150 µg | **610576/77** |
| **Cdc42** | 44 | Purified | Ms $IgG_1$ | Dog, Hu, Ms, Rat | WB | 50/150 µg | **610928/29** |
| **Cdk1 (p34 cdc2)** | 1 | Purified | Ms $IgG_1$ | Hu, Ms, Rat | IF, IHC, IP, WB | 50/150 µg | **610037/38** |
| | A-17 | Purified | Ms $IgG_{2a}$ | Hu, Ms | IP, WB | 0.1 mg | **554161** |
| | Polyclonal | Serum | Rab | Hu, Ms | IP, WB | 0.1 ml | **558900** |
| **Cdk1 Peptide (p34 cdc2, Cdc2 Peptide)** | | Purified | | | Block | 100 µg | **610039** |
| **Cdk1/Cdc2 (pY15), Phospho-Specific** | 44 | Purified | Ms $IgG_1$ | Hu | WB | 50/150 µg | **612306/07** |
| **Cdk1/Cdk2** | AN21.2 | Purified | Ms $IgG_{2a}$ | Hu | WB | 50/150 µg | **551526/25** |
| **Cdk2** | 55 | Purified | Ms $IgG_{2a}$ | Dog, Hu, Ms, Rat | WB, IF, IHC, IP | 50/150 µg | **610145/46** |
| | Polyclonal | Serum | Rab Ig | Hu | WB | 100 µl | **551272** |
| | Polyclonal | Serum | Rab Ig | Hu, Ms | IP, WB | 0.1 ml | **558896** |
| **Cdk5** | G162-9 | Purified | Ms $IgG_{2a}$ | Hu | WB | 0.1 mg | **554090** |
| **Cdk7** | 17 | Purified | Ms $IgG_1$ | Hu | WB | 50/150 µg | **611972/73** |
| | MO-1 | Purified | Ms $IgG_{2b}$ | Hu | IF, IHC(Fr), IP, WB | 0.1 mg | **556345** |
| **Cyclin A** | 25 | Purified | Ms $IgG_1$ | Hu | IF, WB | 50/150 µg | **611268/69** |
| | BF683 | Purified | Ms IgE | Hu | FCM, IP, WB | 250 µg | **554175** |
| | BF683 | Purified | Ms IgE | Hu | FCM, IP, WB | 100 µg | **554174** |
| | BF683 | FITC Set | Ms IgE | Hu | FCM | 100 tests | **554107** |
| | BF683 | PE Set | Ms IgE | Hu | FCM | 100 tests | **550913** |
| | Polyclonal | Purified | Rab Ig | Chick, Hu | IF, IHC, WB | 150 µg | **610230** |
| **Cyclin A1** | B88-2 | Purified | Ms $IgG_1$ | Hu | WB | 100 µg | **556600** |
| **Cyclin B** | 18 | Purified | Ms $IgG_1$ | Hu, Rat | IF, IHC, WB | 50/150 µg | **610219/20** |
| | F2F4 | Purified | Ms $IgG_1$ | Dros | WB | 0.1 mg | **558591** |
| **Cyclin B1** | GNS-1 | Purified | Ms $IgG_1$ | Hu | IHC(Fr), IHC(F)* | 1 ml | **550846** |
| | GNS-11 | Purified | Ms $IgG_{2a}$ | Hu, Ms, Ham | FCM, IF, IP, WB | 250 µg | **554179** |
| | GNS-11 | Purified | Ms $IgG_{2a}$ | Hu, Ms, Ham | FCM, IF, IP, WB | 100 µg | **554178** |
| | Polyclonal | Serum | Rab | Hu | IP, WB | 100 µl | **558899** |
| **Cyclin B1 (a.a 1-21)** | GNS-1 | Purified | Ms $IgG_1$ | Hu, Ms | FCM, IF, IHC(F), IHC(Fr), IP, WB | 250 µg | **554177** |
| | GNS-1 | Purified | Ms $IgG_1$ | Hu, Ms | FCM, IF, IHC(F), IHC(Fr), IP, WB | 100 µg | **554176** |
| | GNS-1 | FITC Set | Ms $IgG_1$ | Hu, Ms | FCM | 100 tests | **554108** |
| | GNS-1 | PE Set | Ms $IgG_1$ | Hu | FCM | 100 tests | **550783** |
| **Cyclin C** | Polyclonal | Serum | Rab | Hu, Ms | IP, WB | 100 µg | **558903** |
| **Cyclin D1** | DCS-6 | Purified | Ms $IgG_{2a}$ | Hu, Ms, Rat | FCM, IHC(F), IHC(Fr), IP, WB | 100 µg | **556470** |
| | DCS-6 | FITC Set | Ms $IgG_{2a}$ | Hu, Ms, Rat | FCM | 100 tests | **556881** |
| | G124-326 | Purified | Ms $IgG_1$ | Hu | FCM, IP, WB | 250 µg | **554181** |
| | G124-326 | Purified | Ms $IgG_1$ | Hu | FCM, IP, WB | 100 µg | **554180** |
| | G124-326 | FITC Set | Ms $IgG_1$ | Hu | FCM | 100 tests | **554109** |
| **Cyclin D1 Recombinant Human Protein** | | | | | WB | 0.5 ml | **554319** |
| **Cyclin D2** | G132-43 | Purified | Ms $IgG_{2a}$ | Hu | IP, WB | 250 µg | **554201** |
| | G132-43 | Purified | Ms $IgG_{2a}$ | Hu | IP, WB | 100 µg | **554200** |
| **Cyclin D3** | 1 | Purified | Ms $IgG_{2b}$ | Dog, Hu, Ms, Rat | IF, IHC, IP, WB | 50/150 µg | **610279/80** |
| | G107-565 | Purified | Ms $IgG_1$ | Hu | FCM, IF, IP, WB | 100 µg | **554195** |
| | G107-565 | FITC Set | Ms $IgG_1$ | Hu | FCM | 100 tests | **554111** |
| **Cyclin E** | HE12 | Purified | Ms $IgG_1$ | Hu | FCM, WB | 50/150 µg | **551159/60** |
| | HE67 | Purified | Ms $IgG_{2b}$ | Hu | IP, IVK | 250 µg | **554193** |
| | HE67 | Purified | Ms $IgG_{2b}$ | Hu | IP, IVK | 100 µg | **554192** |
| **Cyclin F** | B74-2 | Purified | Ms $IgG_1$ | Hu | WB | 100 µg | **556598** |
| **Cyclin G1** | Polyclonal | Serum | Rab | Hu | WB | 200 µl | **550782** |
| **Cyclin H** | G301-1 | Purified | Ms IgM | Hu | WB | 100 µg | **554133** |
| **Cyclins D1/D2/D3** | G124-259 | Purified | Ms $IgG_1$ | Hu | FCM, IP, WB | 100 µg | **554203** |
| | G124-259 | Purified | Ms $IgG_1$ | Hu | FCM, IP, WB | 250 µg | **554204** |
| | G124-259 | FITC Set | Ms $IgG_1$ | Hu | FCM | 100 tests | **554112** |

## p53, p21, p19, and p18

| DESCRIPTION | CLONE | FORMAT | ISOTYPE | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|---|---|
| **p18** | A33-1 | Purified | Ms $IgG_1$ | Ms | IP, WB | 0.1 mg | **556392** |
| **p19** | 3B6 | Purified | Ms $IgG_1$ | Hu, Ms | IP, WB | 0.1 mg | **556426** |
| **p21** | 18A10 | Purified | Ms $IgG_{2b}$ | Hu | IP, WB | 0.1 mg | **554229** |
| | 2G12 | Purified | Ms $IgG_1$ | Hu | IHC(P), IP, WB | 0.1 mg | **554262** |
| | 6B6 | Purified | Ms $IgG_1$ | Hu | IHC(Fr), IHC(F)* | 1 ml | **550833** |
| | 6B6 | Purified | Ms $IgG_1$ | Hu | IHC(Fr), IHC(P), IP, WB | 0.1 mg | **554228** |
| | Polyclonal | Serum | Rab | Ms | IP, IVK, WB | 0.1 ml | **554085** |
| | SX118 | Purified | Ms $IgG_1$ | Hu, Ms, Rat | IF, IHC(Fr), IHC(P), IP, WB | 0.1 mg | **556430** |
| | SX118 | Purified | Ms $IgG_1$, κ | Hu | IHC(Fr), IHC(F)* | 1 ml | **550827** |
| | SXM30 | Purified | Ms $IgG_1$ | Hu, Ms, Rat | IF, IHC(Fr), IHC(P), IP, WB | 0.1 mg | **556431** |
| **p53** | 80 | Purified | Ms $IgG_{2b}$, κ | Dog, Hu | IF, IP, WB | 50/150 µg | **610183** |
| | DO-1 | Purified | Ms $IgG_{2a}$ | Hu, Bov | IHC(F), IHC(Fr), IP, WB | 0.1 mg | **554293** |
| | DO-7 | Purified | Ms $IgG_{2b}$ | Hu, Monk, Bov | FCM, IHC(F), IHC(Fr), IP, WB | 0.1 mg | **554294** |
| | DO-7 | Purified | Ms $IgG_{2b}$ | Hu | IHC(Fr), IHC(F)* | 1 ml | **550831** |
| | DO-7 | Biotin | Ms $IgG_{2b}$ | Hu, Monk, Bov | IHC(F) | 0.5 mg | **554295** |
| | DO-7 | FITC Set | Ms $IgG_{2b}$ | Hu, Monk, Bov | FCM | 100 tests | **554298** |
| | DO-7 | PE Set | Ms $IgG_{2b}$ | Hu, Monk, Bov | FCM | 100 tests | **556534** |
| | G59-12 | Purified | Ms $IgG_1$ | Hu | IHC(Fr), IHC(F)* | 1 ml | **550832** |
| | G59-12 | Purified | Ms $IgG_1$ | Hu, Ms, Rat | IHC(F), IHC(Fr), IP, WB | 0.1 mg | **554157** |
| | G59-12 | PE Set | Ms $IgG_1$ | Hu, Ms, Rat | FCM | 100 tests | **557027** |
| | G59-12 | FITC Set | Ms $IgG_1$ | Hu, Ms, Rat | FCM | 100 tests | **557026** |
| | PAb 122 | Purified | Ms $IgG_{2b}$ | Hu, Ms, Rat, Monk, Ham | FCM, IF, IP, WB | 0.1 mg | **554147** |
| | PAb 1801 | Purified | Ms $IgG_1$ | Hu | IF, IHC(Fr), IHC(P), IP, WB | 0.1 mg | **554169** |
| | PAb 1801 | Purified | Ms $IgG_1$ | Hu | IF, IHC(Fr), IHC(P), IP, WB | 0.25 mg | **554170** |
| | PAb 240 | Purified | Ms $IgG_1$ | Hu, Ms, Rat, Ham, Monk, Bov, Chick | IHC(Fr), IP, WB | 0.1 mg | **554166** |
| | PAb 240 | Purified | Ms $IgG_1$ | Hu, Ms, Rat, Ham, Monk, Bov, Chick | IHC(Fr), IP, WB | 0.25 mg | **554167** |

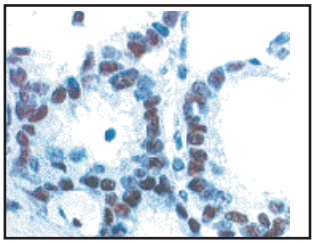


*Figure 35. Anti-human p53, (Cat. No. 554294). Formalin-fixed, paraffin-embedded tissue section of human breast carcinoma stained with anti-human p53 (clone DO-7) using a DAB chromogen and Hematoxylin counterstain.*

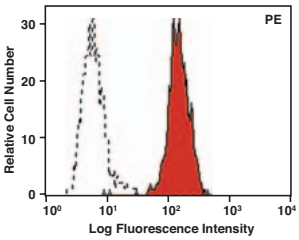



*Figure 36. Profile of permeabilized HT-29 colon adenocarcinoma cells analyzed on a FACScan™ (BDIS, San Jose, CA). Cells were stained with anti-p53 PE (clone G59-12) or with an $IgG_1$ isotype control (Cat. No. 557027).*

## Retinoblastoma Protein (Rb)

| DESCRIPTION | CLONE | FORMAT | ISOTYPE | REACT | APPS | SIZE | CAT. NO. |
|---|---|---|---|---|---|---|---|
| **Rb (a.a. 1-240)** | G99-2005 | Purified | Ms $IgG_1$ | Hu | IP, WB | 0.1 mg | **554162** |
| **Rb (a.a. 300-380)** | C36 | Purified | Ms $IgG_1$ | Hu, Chick | GS, IHC(Fr), IP, WB | 0.1 mg | **554142** |
| **Rb (a.a. 300-380)** | G3-349 | Purified | Ms $IgG_1$ | Hu | IP, WB | 0.1 mg | **554140** |
| **Rb (a.a. 300-380)** | G4-340 | Purified | Ms $IgG_1$ | Hu | IP, WB | 0.1 mg | **554141** |
| **Rb (a.a. 300-508)** | 2 | Purified | Ms $IgG_{2a}$ | Chick, Hu | IF, WB | 50/150 µg | **610884** |
| **Rb (a.a. 332-344)** | G3-245 | Purified | Ms $IgG_1$ | Ms, Rat, Hu, Monk, Qua, Mink | FCM, GS, IF, IHC(F), IP, WB | 0.1 mg | **554136** |
| **Rb (a.a. 332-344)** | G3-245 | Purified | Ms $IgG_1$ | Ms, Rat, Hu, Monk, Qua, Mink | IHC(Fr) | 1 ml | **550830** |
| **Rb (a.a. 332-344)** | G3-245 | FITC Set | Ms $IgG_1$ | Ms, Rat, Hu, Monk, Qua, Mink | FCM | 100 tests | **556538** |
| **Rb (a.a. 332-344)** | G3-245 | PE Set | Ms $IgG_1$ | Ms, Rat, Hu, Monk, Qua, Mink | FCM | 100 tests | **556539** |
| **Rb (a.a. 393-572)** | XZ104 | Purified | Ms $IgG_1$ | Hu, Ms, Chick | IP | 0.1 mg | **554143** |
| **Rb (a.a. 443-622)** | XZ55 | Purified | Ms $IgG_1$ | Hu, Ms, Chick, Xen, Qua | GS, IP, WB | 0.1 mg | **554144** |
| **Rb (a.a. 444-535)** | XZ91 | Purified | Ms $IgG_{2a}$ | Hu, Chick, Mink | IF, IP, WB | 0.1 mg | **554145** |
| **Rb (a.a. 622-665)** | XZ133 | Purified | Ms $IgG_1$ | Hu, Ms, Chick | IP | 0.1 mg | **554146** |
| **Underphosphorylated Rb** | G99-549 | Purified | Ms $IgG_1$ | Hu | IP, WB | 0.1 mg | **554164** |
| **(a.a. 514-610)** | G99-549 | FITC Set | Ms $IgG_1$ | Hu | FCM | 100 tests | **550501** |
| | G99-549 | PE Set | Ms $IgG_1$ | Hu | FCM | 100 tests | **550502** |

## ADME/TOX

During the development of a new drug, it is important to investigate the potential adverse effects the drug might have. Prior to clinical testing of a drug candidate on patients, toxicology studies are conducted in cell lines or in laboratory animals, primarily rats, dogs, and non-human primates.

BD Biosciences – Discovery Labware combines expertise and state-of-the-art toxicity detection technologies to help identify better, safer drugs faster.

Toxicity testing using BD Gentest™ hepatocytes and cell lines expressing P450 enzyme toxicity is a critical area of concern for drug candidates. Compounds may exhibit intrinsic toxicity or must undergo metabolic activation by cytochrome P450 or other enzymes to manifest toxicity.

Hepatotoxicity is a major cause of drug candidate failure, both pre- and post-market launch. Two tests for the potential of drug candidate-induced hepatocyte toxicity are available: 1) measurement of ATP content and 2) leakage of lactate dehydrogenase (LDH).[1] Both tests are conducted using hepatocytes, the "gold standard" for analyzing toxicity *in vitro*. These tests serve as independent means to determine cytotoxicity with cell viability as the endpoint.

In both tests, the concentration of drug candidate needed to cause 50% cell toxicity/death to human hepatocytes is determined. The number and spacing of drug concentrations are flexible for these tests. BD Biosciences also offers testing using hepatocytes from other preclinical species.

For complete list of products and services for BD Gentest™, please go to the web site: **www.bdbiosciences.com/discovery_labware/gentest/products/**

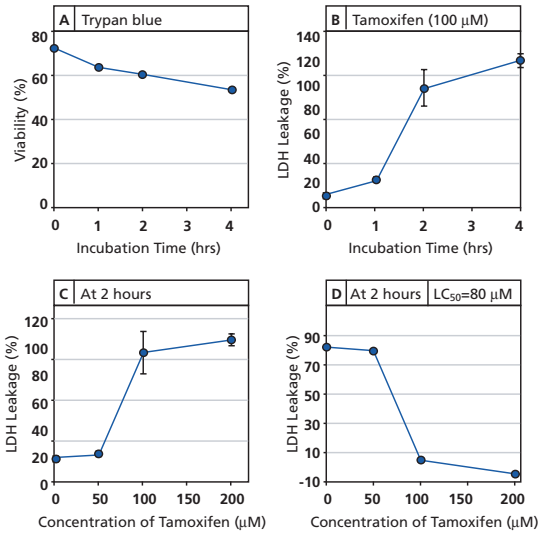



***Figure 37. Tamoxiten-induced leakage of lactate dehydrogenase (LDH) from hepatocytes in suspension.*** *BD Gentest™ Cryopreserved Hepatocytes were thawed and purified. The cells were then incubated with tamoxifen in a 24-well plate. The activity of LDH in medium was determined at different periods of incubation. The data are the mean ± SD of three separate wells. A: Control viability determined by trypan blue; B: Time-response; C: Concentration- response; D: Cell viability and $LC_{50}$ calculated from concentration-response.*

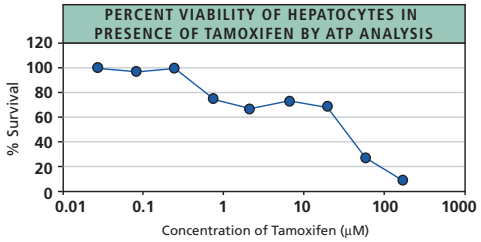



*Figure 38. BD Gentest™ Cryopreserved Human Hepatocytes were thawed and purified using the BD Gentest Cryopreserved Hepatocyte Purification Kit (Cat. No. 454500). Cells were resuspended in MEM and incubated with tamoxifen over a four-hour time period. The concentration of ATP was determined using a chemiluminescent assay (Perkin Elmer). The representative data are the mean of four separate wells. The experiment was performed in triplicate.*

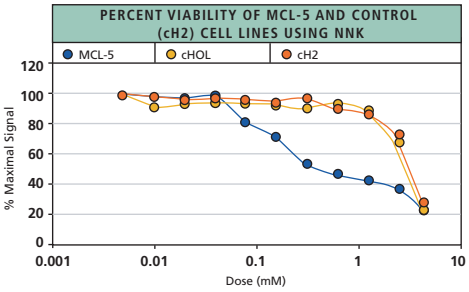



*Figure 39. The $IC_{50}$ values calculated for MCL-5 cells by linear interpolation was determined to be 150 nM NNK. The cH2 cells demonstrated a 30% inhibition at 480 nM NNK whereas the $IC_{50}$ values for control cells were >200 µM.*

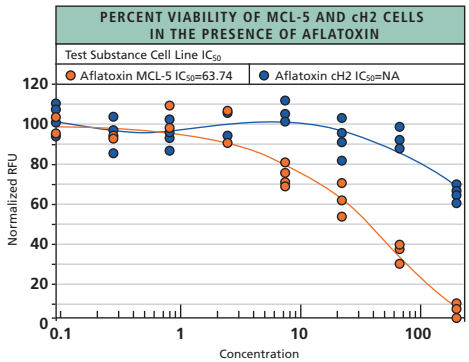



*Figure 40. The $IC_{50}$ value was generated using the BD Gentest™ MPM/ADMET Software Program and a 4-parameter fit model. The cH2 cells demonstrated 20% inhibition at 200 nM aflatoxin after 72 hours of exposure*

## Toxicity Testing in a Human Cell Line Engineered to Express P450

The BD Gentest™ Metabo-Tox Assay Kit is designed to assess P450-mediated toxicity. Using our MCL-5 cell line that expresses five human P450 enzymes (CYP 1A1, 1A2, 3A4, 2A6, and 2E1) and epoxide hydrolase, BD Biosciences has created a toxicity assay that is consistent over time and also avoids the variations in P450 expression that occur when using human hepatocytes.[2] The human P450s incorporated into the MCL-5 cell line are the major P450s implicated in creating toxic metabolites. The MCL-5 assay can differentiate between parent and metabolite toxicity in one assay by comparing IC50 values with the control cell line cH2, which does not contain the transfected genes.

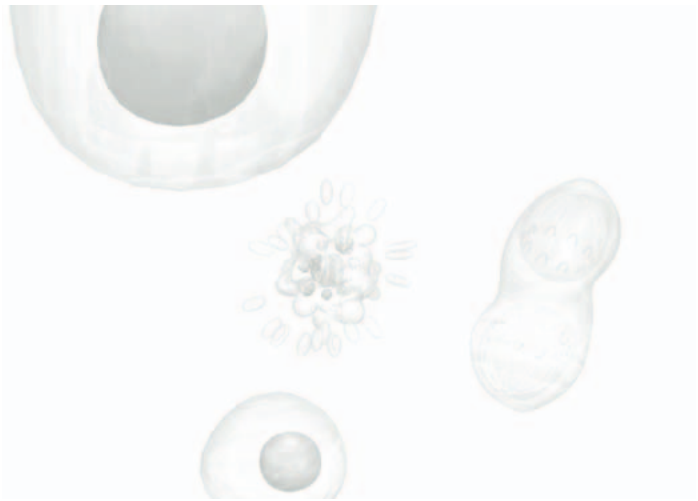
The kit measures oxygen respiration as an indicator of cell viability,[3] although other assays such as ATP detection are available. The number and spacing of drug concentration is flexible.

*In vitro* techniques based on cDNA-expressed enzymes, human tissue and cells, allow researchers to screen for drug-drug interactions, metabolic stability, and bioavailablity early in the drug development process. BD Gentest™ Contract Research Services combine our expertise and innovative products to help you identify better drugs faster. We offer many contract services that help the sponsor address important drug metabolism concerns during the drug discovery and development processes. Many of these tests can be performed in accordance with Good Laboratory Practices (GLP) regulations.

For complete information regarding BD Gentest products and services, please visit the web site:
**www.bdbiosciences.com/discovery_labware/gentest/products**

**References**


1. Tirmenstein M.A., *et al*. Glutathione depletion and the production of reactive oxygen species in isolated hepatocyte suspensions. *Chemico-Biological Interactions.*127:201 (2000).
2. Crespi, C.L., *et al*. A metabolically competent human cell line expressing five cDNAs encoding procarcinogenactivating enzymes: application to mutagenicity testing. *Chem. Res. Toxicol*. 4:566 (1991).
3. Wodnicka, N., *et al*., Novel fluorescent technology platform for high throughput cytotoxicity and proliferation assays. *J. Biomol. Screen*. 5:141 (2000).

## Products and Services

| DESCRIPTION | CAT. NO. |
|---|---|
| MCL-5 Metabo-Tox Assay Kit* | 459600 |

* Product of BD Biosciences – Discovery Labware. Please order accordingly.

## Contract and Research Services

| DESCRIPTION | CAT. NO. |
|---|---|
| Hepatocyte Toxicity* | inquire |
| Tosxicity Testing in a Human Cell Line Engineered to Express P450* | inquire |

* Product of BD Biosciences – Discovery Labware. Please order accordingly.

## Summary of IC50 Values for Metabolism-dependent Toxins

| TEST SUBSTRATE | CHOL CELLS | cH2 CELLS | MCL-5 CELLS | MAJOR P450'S |
|---|---|---|---|---|
| Benzopyrene | 8% toxicity > 20 µM | 8% toxicity > 20 µM | 1.6 µM | 1A1, 3A4, 1B1 |
| Aflatoxin | 20% toxicity > 200 µM | N/D | 47.9 µM | 3A4, 1A2, 1A1 |
| NNK | 25% toxicity > 480 µM | 29% toxicity > 480 µM | 153.5 µM | 2A6, 2E1, 2D6 |
| Dibenzopyrene | 10% toxicity > 200 µM | N/D | 2.27 µM | 1A1, 3A4, 1A2 |
| Acetaminophen (50 µM BSO pretreated) | 2.48 mM | 2.9 mM | 1.2 mM | 2E1, 3A4, 1A2 |
| PhIP | 110.3 µM | 111.2 µM | 21.28 µM | 1A1 |
| Cyclophosphamide | 5.4 mM | 5.5 mM | 2.83 mM | 2B6, 3A4 |

## Genotoxicology

### DNA Damage Antibodies

Cellular DNA is continually exposed to a wide variety of exogenous and endogenous factors that can alter the DNA sequence. One estimate indicates our cells are subjected to several thousand DNA alterations per day. For this reason, a variety of DNA repair pathways have evolved to protect cells and to maintain genomic stability.

Mammalian cells can take advantage of a variety of DNA repair pathways, which include base excision repair (BER), mismatch repair, recombinational repair, postreplication repair, and nucleotide excision repair (NER). Deficiencies in these DNA repair pathways are implicated in the pathogenesis of various human diseases and conditions, such as cancer and aging. This may be due to the fact that these DNA repair pathways interact with a number of cell signaling pathways, and are involved in cell cycle regulation and transcription.

One mechanism whereby eukaryotic cells respond to DNA damage is the synthesis of poly ADPribose (PAR). PAR is synthesized after activation of the DNA repair enzyme poly (ADP-ribose) polymerase (PARP). Upon DNA damage, PARP binds to DNA strand breaks and catalyzes the addition of long branched chains of PAR to a number of nuclear proteins, including topoisomerases, histones, and PARP. The amount of PAR formed in living cells with DNA damage is commensurate with the extent of the damage.

A recent study demonstrated the presence of a PAR-binding sequence in a number of molecules that are characterized as DNA damage checkpoint proteins including p53, p21CIP1/WAF1, xeroderma pigmentosum group A complementing protein, MSH6, DNA ligase III, XRCC1, DNA polymerase e, DNA-PKCs, Ku-70, NF-κB, inducible nitric oxide synthase, caspase-activated DNase, and telomerase.

BD Biosciences offers a comprehensive selection of antibodies and reagents which can be used in a variety of applications to evaluate the processes involved in DNA damage and repair.

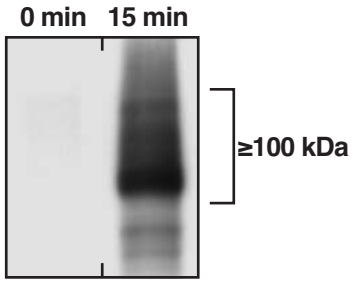



*Figure 41. Western blot analysis of PAR. Lysates from HeLa cells that were either left untreated (0 min) or treated with $H_2O_2$ (15 min), washed in PBS, and probed with anti-poly ADP-ribose (L96-10). PAR identifies bands greater than 100 kDa.*

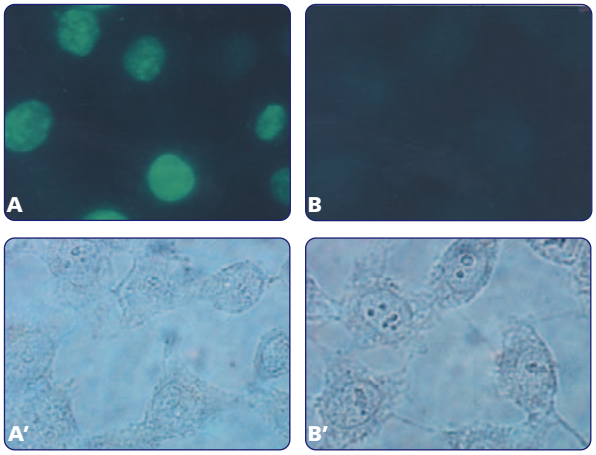



*Figure 42. Immunofluorescence staining of PAR. HeLa cells were either treated with $H_2O_2$ [A] or left untreated [B], washed in PBS, and fixed in MEOH/acetone. Following fixation, cells were permeabilized and then stained with purified anti-poly ADP-ribose (clone 10H, Cat. No. 550781). Cells were washed in PBS and incubated with FITC-conjugated goat anti-mouse secondary antibody (Cat. No. 554001). Cells were then washed 5× in PBS and visualized by immunofluorescence microscopy. A' and B' represent phase contrast correlates of A and B, respectively.*

## DNA Damage and Repair Antibodies Product List

| SPECIFICITY | CLONE | APPLICATION | SIZE | CAT. NO. |
|---|---|---|---|---|
| **AOX1** | 7 | WB | 50/150 µg | **611494/95** |
| **BRCA-1** | 42 | WB | 50/150 µg | **611842/3** |
| **BRCA-1** | Polyclonal | IP, WB | 100 µl | **556443** |
| **BRCA-1** | Polyclonal | IP, WB | 100 µl | **556445** |
| **BrdU** | 3D4 | FC | 100 µg | **555627** |
| **BrdU FITC Set** | 3D4 | FC | 100 tests | **556028** |
| **BrdU PE Set** | 3D4 | FC | 100 tests | **556029** |
| **Cdc25C** | C2-2 | WB | 100 µg | **556576** |
| **Cdc25C** | TC113 | WB, IP, IF | 50/150 µg | **550920/1** |
| **Cdc25C** | TC14 | WB, IP | 50 µg | **550922** |
| **CHD3** | 44 | WB, IF | 50/150 µg | **611846/7** |
| **Chk2** | 19 | WB, IF | 50/150 µg | **611570/1** |
| **DNA Ligase III** | 7 | WB | 50/150 µg | **611876/7** |
| **DNA-PK (p350)** | 4F10C5 | IP, WB | 100 µg | **556456** |
| **DNA-PKcs (p350)** | 6 | WB, IHC, IF | 50/150 µg | **610804/5** |
| **DNA Polymerase ∂** | 22 | WB, IF | 50/150 µg | **610972/3** |
| **DNA Polymerase e Catalytic** | 34 | WB, IF | 50/150 µg | **611238/9** |
| **DNA Topoisomerase I** | C-21 | WB | 100 µg | **556597** |
| **ERCC1** | 8F1 | WB | 100 µg | **556452** |
| **FEN-1** | 21 | WB, IF | 50/150 µg | **611294/5** |
| **hHR23B** | 16 | WB, IF | 50/150 µg | **611018/9** |
| **Ku-70** | 15 | WB, IF | 50/150 µg | **611892/3** |
| **Ku-70/80 Heterodimer** | 162 | IP, FC, E | 100 µg | **556460** |
| **Ku-80** | 111 | IP, WB, IF, IHC | 100 µg | **556429** |
| **Ku-80** | 7 | WB, IF | 50/150 µg | **611360/1** |
| **MDM2** | SMP14 | IP, WB | 100 µg | **556353** |
| **MGMT** | MT5.1 | IP, WB, FC, IHC | 100 µg | **557045** |
| **MLH1** | G168-728 | IP | 100 µg | **554073** |
| **Mre11** | 18 | WB, IF | 50/150 µg | **611366/7** |
| **MSH2** | G219-1129 | WB, IHC | 100 µg | **556349** |
| **MSH2** | 27 | WB, IF | 50/150 µg | **610360/1** |
| **MSH3** | 52 | WB, IF | 50/150 ug | **611390/1** |
| **MSH6/GTBP** | 44 | WB, IF | 50/150 µg | **610918/9** |
| **NBS1** | 34 | WB, IF | 50/150 µg | **611870/1** |
| **PAR** | 10H | IF | 50 µg | **550781** |
| **PARP** | C2-10 | WB, IF | 100 µl | **556362** |
| **PARP** | 4C10-5 | WB, FC, IP | 100 µg | **556494** |
| **PARP** | 42 | WB, IF | 50/150 µg | **611038/9** |
| **PCNA** | PC10 | IHC, IF, FC | 100 µg | **555566** |
| **PCNA FITC Set** | PC10 | FC | Kit | **556030** |
| **PCNA PE Set** | PC10 | FC | Kit | **556031** |
| **PCNA** | 24 | WB, IHC, IF | 50/150 µg | **610664/5** |
| **PMS2** | A16-4 | IP, WB | 100 µg | **556415** |
| **PMS2** | 37 | WB | 50/150 µg | **610338/9** |
| **Rad50** | 13 | WB, IF | 50/150 µg | **611010/1** |
| **hRad9** | 56 | WB, IF | 50/150 µg | **611324/5** |
| **RCC1** | 9 | WB, IF, IHC | 50/150 µg | **610377/8** |
| **Ref-1** | 15 | WB, IF | 50/150 µg | **610816/7** |
| **RNase HI** | 30 | WB, IF | 50/150 µg | **611356/7** |
| **Superoxide Dismutase** | G215-1 | WB | 100 µg | **556360** |
| **TopBP1** | 33 | WB, IF | 50/150 µg | **611874/5** |
| **TRF2** | 36 | WB, IF | 50/150 µg | **611200/1** |
| **WRN** | 30 | WB, IF | 50/150 µg | **611168/9** |
| **XPA** | 12F5 | IP, WB | 100 µg | **556453** |
| **XPD** | 19 | WB, IF | 50/150 µg | **611828/9** |
| **XRCC4** | 4 | WB, IF | 50/150 µg | **611506/7** |

## µicroFlow® Micronucleus Analysis Kits

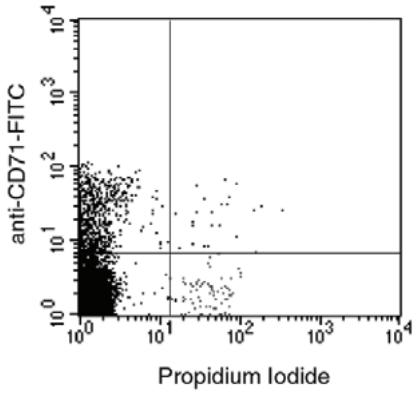
- Ideal for pre-IND and drug discovery
- High-throughput analysis of cytogenetic damage *in vivo*
- Biological standards are included to guide instrument set–up
- Easy-to-use kit format
- Fast: get results within seven days using the µicroFlow$^{BASIC}$ service, or analyze 20,000 RETs per sample in just minutes with µicroFlow$^{PLUS}$

The micronucleus assay is an established method for studying *in vivo* chromosomal damage. It is based on the observation that displaced chromatin, resulting from chromosome loss or breakage, may fail to be incorporated into daughter nuclei as a cell divides. The resulting "micronucleus" is found in the cytoplasm. Elevations in the frequency of micronuclei (MN) are indicative of genotoxic activity.

The µicroFlow® Micronucleus Analysis Kits are designed to measure MN events in peripheral blood cells of rodents. During erythropoiesis, an erythroblast expels its main nucleus to become a reticulocyte (RET), while the MN remain in the cytoplasm. The newly formed RET is then released from the bone marrow into the circulating bloodstream, where it develops into a normochromatic erythrocyte (NCE). The µicroFlow® kits are based on a RET-specific cell surface marker, CD71, which differentially labels immature erythrocytes. When fixed peripheral blood cells are appropriately treated, NCEs and RETs with and without MN are easily resolved and quantified by flow cytometry. These kits measure the frequency of each cell population of interest (%RET, %MN-RET, %MN-NCE).

µicroFlow$^{BASIC}$ and µicroFlow$^{PLUS}$ kit utilize flow cytometry, which enables the high throughput analysis of many more cells per sample relative to microscopy-based MN scoring. The result is greater precision, higher sensitivity, and a quicker turn-around time.

The µicroFlow$^{PLUS}$ kit provides the reagents required to collect, fix, and analyze mouse or rat blood in your laboratory, while the µicroFlow$^{BASIC}$ kit provides the reagents needed to collect, fix, and ship mouse or rat blood samples to Litron Laboratories for evaluation (customers are provided with all raw data and calculated cell population frequencies).



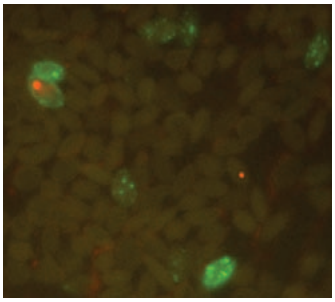



*Figure 43. FITC and PI staining of mouse erythrocytes for flow cytometric analysis*

| DESCRIPTION | SIZE | CAT. NO. |
|---|---|---|
| Mouse µicroFlow$^{PLUS}$ Kit | 60 Tests | 552730 |
| Rat µicroFlow$^{PLUS}$ Kit | 60 Tests | 552731 |
| Mouse µicroFlow$^{BASIC}$ Kit | 60 Tests | 552728 |
| Rat µicroFlow$^{BASIC}$ Kit | 60 Tests | 552729 |

## Immunotoxicology

Immunotoxicology studies adverse health effects resulting from xenobiotic (drug/chemical that is not native to the mammalian system) interactions with the immune system.[1-2]

There are five major areas of immunotoxicology as defined by the US FDA guidelines: immunosuppression, immunogenicity, hypersensitivity, autoimmunity, and adverse immunostimulation. Chemistry values and histopathology results routinely provide the first evidence that a drug has an immunological effect.[3]

If initial studies identify signs of immunosuppression, it is recommended that additional tests be conducted to determine the potential mechanism. Cell phenotyping and enumeration is an easy and powerful tool to quickly identify changes in specific leukocyte levels. A summary of markers used to identify different cell types by flow cytometry is shown in the table below.


**References**

1. Toxicologic Pathology, 2002. 30(1): p. 54-58.
2. Toxicology Letters, 1998. 102-103: p. 267-270.
3. Toxicology, 1994. 86(3): p. 187-212.


## Immunotoxicology. Cell Phenotype Markers

| | Rat | | Dog | | Non-Human Primate | |
|---|---|---|---|---|---|---|
| | SPECIFICTY | CLONE | SPECIFICTY | CLONE | SPECIFICTY | CLONE |
| **T cell marker** | CD3 | 1F4 | Pan T | LSM8.358 | CD3 | SP34.2 |
| **T cell subset marker** | CD4 | OX-38, OX-35 | CD4 | LSM12.125 | CD4 | M-T477 |
| **T cell subset marker** | CD8a | OX-8 | CD8 | LSM1.140 | CD8 | RPA-T8 |
| **B cell marker** | CD45RA | OX-33 | Pan B | LSM11.425 | CD20 | 2H7 |
| **NK cell marker** | NKR-P1A | 10/78 | NK cell marker | DL10* | CD56 | MY31 |
| **Monocyte marker** | CD11b | WT.5 | CD11b | CA16.3E10* | CD14 | M5E2 |
| **Granulocytes marker** | Granulocytes | HIS48 | CD11b | CA16.3E10* | CD11b | ICRF44 |
| **T cell activation marker** | CD25 | OX-39 | Activation marker | CTL 2.58 | CD25 | M-A251 |
| **B cell activation marker** | CD80 | 3H5 | Activation marker | CTL 2.58 | CD86 | 2331(FUN-1) |
| **Adhesion receptor** | CD29 | Ha2/5 | CD49d | CA.4.5B6* | CD29 | MAR4 |
| **Adhesion receptor** | CD62L | HRL1 | CD54 | CL18.1D8* | CD62L | SK11 |

* Not available from BD Biosciences

## Rat and Canine C-Reactive Protein ELISA Kits

BD Biosciences – Pharmingen now offers ELISA kits to quantitatively measure C-reactive protein levels in rat (rCRP) and canine (cCRP). Each kit contains an ELISA standard (serum with elevated levels of CRP), a capture antibody-coated 96-well plate matched with an HRP-conjugated affinity purified detection antibody, substrate/TMB chromogen, and wash buffer. A standard curve can be constructed for accurately measuring the level of CRP in rat or canine serum, plasma, or other fluids and tissues in a total assay time of 30-90 minutes. These test kits can serve as part of a serum protein and cytokine/chemokine analysis panel in many research applications.

### Contents:

- Plate-based assay, 96 wells with 12 break-away strips
- Cost-effective and user-friendly
- High specificity — no cross-reactivity between species
- Break-away wells allow low-volume testing
- One year stability and reproducibility of results
- Easy-to-follow instructions
- Color-coded reagents in convenient, easy-to-use vials

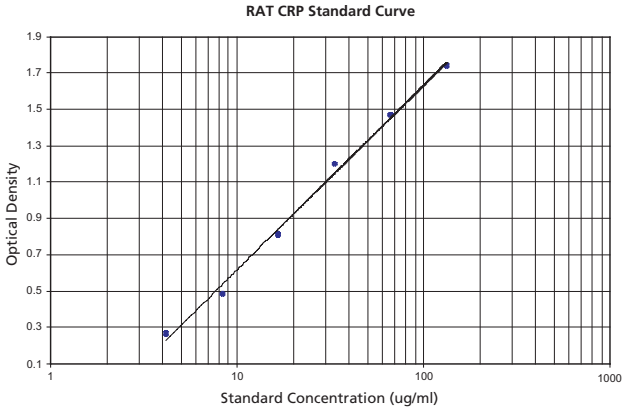



*Figure 44. Standard Curve used in the measurement of rat CRP in serum*

## New BD™ ELISA Kits

| DESCRIPTION | ASSAY RANGE | SIZE | CAT. NO. |
|---|---|---|---|
| **Rat CRP*** | 4.2 - 133.3 µg/ml | 1 Plate | **557825** |
| **Canine CRP*** | 1.3 - 40.0 µg/ml | 1 Plate | **557826** |

*Original equipment manufacturer (OEM) from Helica Biosystems, Inc.

# Toxicology

## B lymphocytes

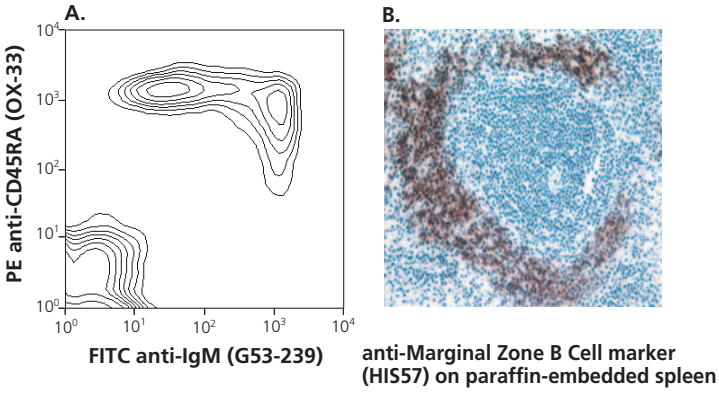



*Figure 45. Flow cytometric analysis of rat splenocytes using PE anti-CD45RA (OX-33) and FITC anti-IgM (G53-239) (A) and immunohistochemical staining of rat spleen with anti-marginal zone B cell marker (H1S57) (B).*

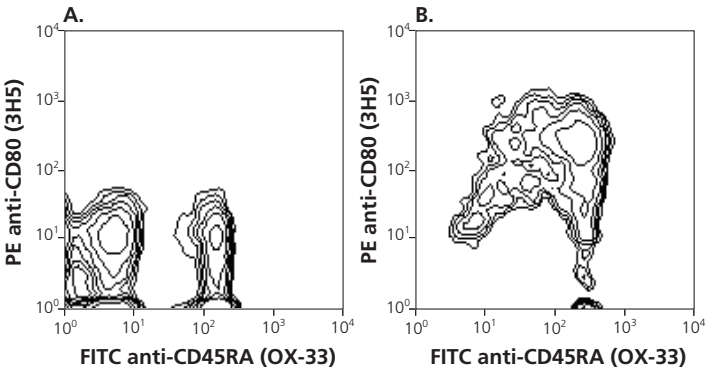



*Figure 46. Activation-induced changes in the level of expression of the CD80 antigen on fresh rat splenocytes (A) and cells treated in vitro for 72 hours with lipopolysaccharide (LPS) (B). Cells were stained simultaneously with FITC anti-CD45RA (OX-33) and PE anti-CD80 (3H5).*

## T lymphocytes

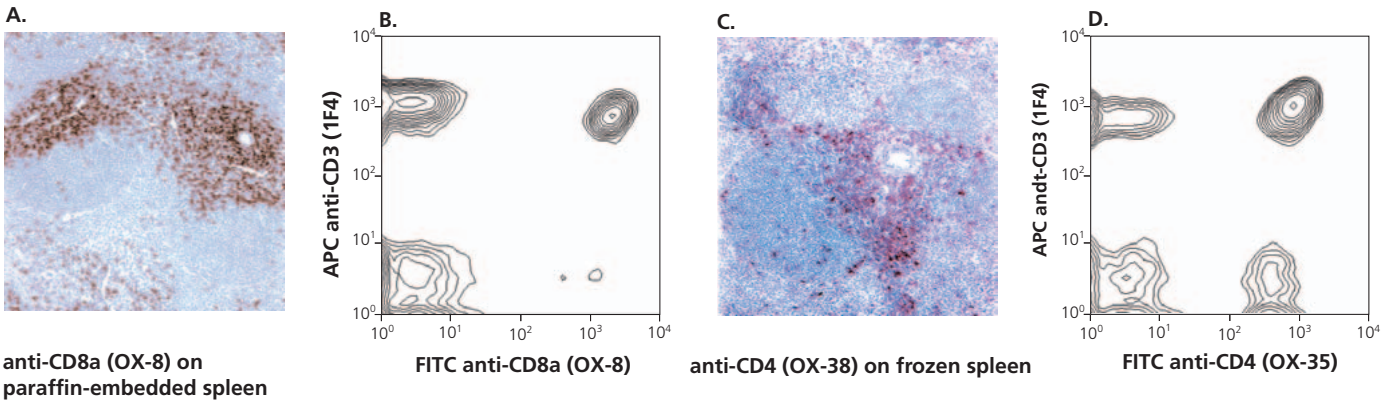



*Figure 47. Immunohistochemical staining of rat tissue with anti-CD8a (A) or anti-CD4 (C) and flow cytometric analysis of rat peripheral blood cells stained with the APC anti-CD3 (1F4) and FITC anti-CD8a (OX-8) (B) or FITC anti-CD4 (OX-35) (D).*

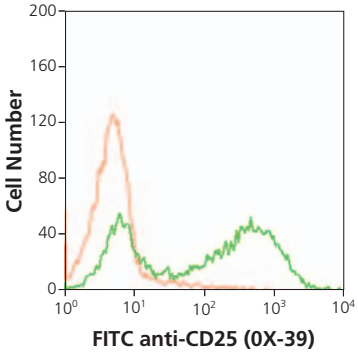



*Figure 48. Expression of CD25 (IL-2Rα chain) on rat spleen T cells ($CD3^+$) following in vitro incubation for 48 hours with medium alone (red line) or activation for 48 hours with Concanavalin A (green line).*

## BD™ Pathway Live Cell Confocal Bioimager (New)

- Proven high-throughput assays for numerous applications including cytotoxicity, apoptosis, translocation, and cell cycle studies
- Fully automated sample processing
- Robust, integrated software with add-in modules for specific applications

The BD™ Pathway Bioimager provides real-time confocal kinetic imaging in an easy-to-use compact unit.

### Integrated System

The BD™ Pathway Bioimager enables cell-based assays of multiple events within living cells, revealing significantly more information about cellular mechanisms and structure. With the ability to image multiple fluorescent dyes in living cells, one can pursue experiments that were previously not possible. The BD Pathway Bioimager can be operated in a fully automated mode using imaging software that seamlessly integrates imaging, liquid handling, plate navigation, and image analysis.

### Rapidly Develop Cell-based Assays

The software included with the BD Pathway Bioimager employs a novel hierarchical data classification algorithm that allows greater flexibility in assay design and implementation. It allows you to configure and analyze many different cell-based assays including translocation, ion kinetics, apoptosis, cyctotoxicity, cell proliferation, and cell cycle experiments. Other specific applications such as neurite outgrowth are available separately as add-ins.

To find out how the BD™ Pathway Bioimager can accelerate your research, visit **www.bdbiosciences.com/pathway**.

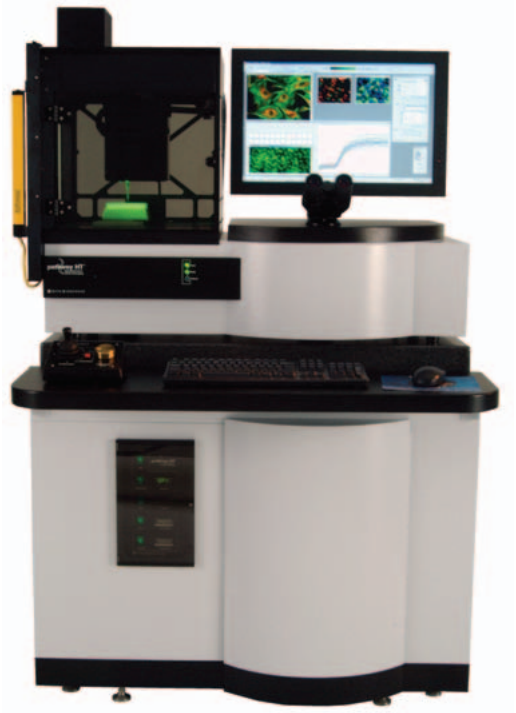


*Figure 49. The BD™ Pathway is a compact, fully automated confocal imaging platform with integrated liquid handling, temperature, and $CO_2$ regulation.*

### Features

- True confocal real-time imaging
- Full-spectrum laser-free illumination
- Auto focus, auto-cell identification, and tracking
- Confocal and wide field viewing modes
- Integrated temperature and $CO_2$ control
- Capable of imaging both cells and tissues
- Binocular eyepiece for direct sample viewing
- Kinetic or endpoint measurement assays
- Multiple plate and slide configurations including 96-well, 384-well, chamber slides, and microscope slides
- Versatile liquid handling and mixing
- Motorized high-resolution X, Y, Z
- Proprietary motionless stage with mobile optics

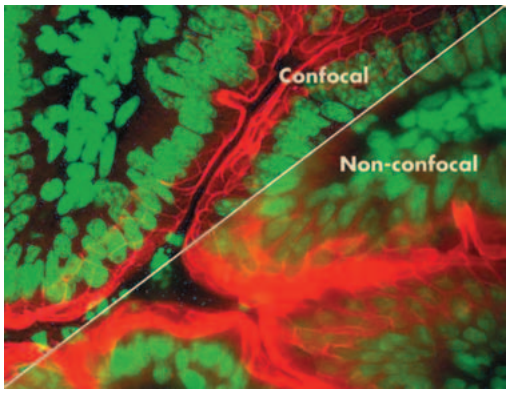



*Figure 50. The BD™ Pathway Bioimager employs a unique laser-free confocal device that improves resolution and allows three-dimensional imaging.*

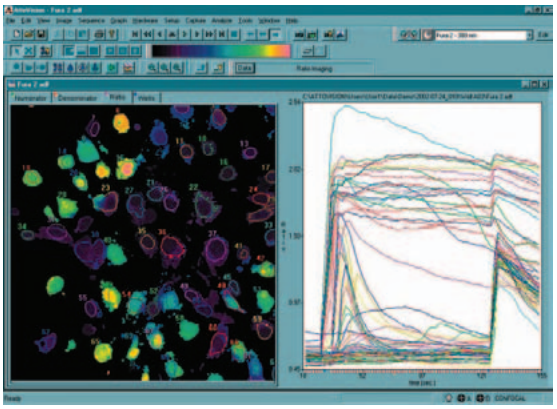


*Figure 51. The BD™ Pathway software integrates a novel data classification capability to provide researchers flexibility in designing and implementing cell- based assays. After imaging at high resolution, cells are automatically identified and fluorescence intensity is monitored at the cellular or subcellular level. Using selective criteria, each cell can be automatically classified during the experimental run using a number of cell-specific features (eg, fluorescence intensity, rate of change, redistribution, etc.). Once classified, cells can be counted and traces color-coded. Additionally, wells can be color-coded based on criteria selected by the user, enabling whole plate maps to be identified by particular responses of interest. The software also incorporates sophisticated 3D rendering capabilities allowing the system to be used as an automated confocal imaging workstation.*

# BD Flow Cytometry Instrumentation

## BD FACSArray™ Bioanalyzer

The BD FACSArray™ bioanalyzer provides researchers with a new and compelling platform capable of analyzing cellular and BD™ Cytometric Bead Array assays. Supported by over 1,000 BD Biosciences products, the bioanalyzer is designed for multiparameter analysis of proteins in immunology and cell biology applications. The system is compact, easy-to-use, and particularly well-suited for BD Cytometric Bead Array (CBA) applications.

Sampling speed is optimized by combining a new plate loader technology for sample input and digital electronics for acquisition rates of up to 15,000 events per second. An entire 96-well plate can be turned around in less than 35 minutes, while saving 1000 events per sample well. Each event contains information for up to six parameters. Featuring a dual-laser system, the bioanalyzer allows the use of several extremely bright fluorophores in parallel, thus enabling applications with a wide dynamic range. The BD FACSArray bioanalyzer offers a powerful, yet easy-to-use solution for many avenues of life sciences research. More information can be found online at **www.bdbiosciences.com/bdfacsarray.**


*Class 1 (I) Laser Product*

| DESCRIPTION | | CAT. NO. |
|---|---|---|
| **BD FACSArray bioanalyzer*** | | inquire |
| ***(see page 44)*** | • Fast microtiter plate sampler | |
| | • Six-parameter detection (Two scatter and four fluorescences) | |
| | • Intuitive software | |
| | • Digital signal processing with up to 15,000 events per second | |
| | • Compact, affordable benchtop unit | |
| **BD FACSCalibur flow cytometry system*** | | inquire |
| | • The industry standard in dual-laser, six-parameter, four-color flow cytometric analysis | |
| | • Includes BD CellQuest Pro Software | |
| | • BD FACSCalibur and BD CellQuest Pro are for *In Vitro* Diagnostic Use when used with IVD cleared assays | |
| **BD FACSCanto benchtop flow cytometry system*** | | inquire |
| | • Dual-laser instrument with true 6-color capability | |
| | • Powerful digital software to make multicolor experimentation fast and simple. | |
| | • Resolves the dimmest events by increased sensitivity | |
| **BD LSR II flow cytometry system*** | | Inquire |
| | • Incorporating digital electronics for use with up to four fixed-alignment lasers (488 nm, 638 nm, 405 nm, and UV) | |
| | • Detect 18 colors through a revolutionary new optical design | |
| | • Flexible and modular for future upgrades | |
| **BD FACSAria cell sorting system*** | | inquire |
| | • First benchtop high-speed sorter with fixed-alignment cuvette flow cell | |
| | • Cuvette flow cell for superior fluorescence sensitivity | |
| | • Up to three air-cooled lasers at 488-nm, 633-nm, and 407-nm wavelengths | |
| | • Digital acquisition rates of up to 70,000 events/second | |
| | • Multicolor analysis of up to 15 parameters | |
| | • Two- and four-way bulk sorting devices for a variety of tube sizes | |
| | • Optional BD Automated Cell Deposition Unit (ACDU) for sorting to BD Multiwell plates or microscope slides | |

*Class 1 (I) Laser Product

## BD RiboQuant™ Multi-probe RNAse protection assay

The BD RiboQuant™ Multi-Probe RNAse protection assay (RPA) can be used to measure mRNA levels of molecules participating in apoptosis, during development, homeostasis, and in many disease conditions. RPA is a method for detecting and quantifying the expression of multiple genes in a single RNA sample. This assay system involves the hybridization of the RNA of interest to their complementary antisense RNA probes. Each individual apoptosis, cell cycle, tumor suppressor, and stress protein gene specific template has been assembled into relevant sets to be used by investigators for the T7 RNA polymerase-directed synthesis of a high-specific activity, [$\alpha$-$^{32}$P]- labeled or biotin labeled, anti-sence RNA probe set. In each set, we have included the L32 gene (which codes a ribosomal gene) and the GAPDH gene (glyceraldehyde 3-phosphate dehydrogenase enzyme involved in glycolysis) to serve as housekeeping gene controls. Following transcription using the BD RiboQuant *In vitro* transcription kit, radioactive (Cat. No. 556850) and non-radioactive (Cat. No. 551917), the probe and total target RNA are solution-hybridized overnight using the reagents included in the RPA kit (Cat. No. 556134). Subsequently, free probe and single stranded non-protected RNA molecules are digested with a pre-optimized mixture of RNAse A and T1. The remaining RNAse-protected probes are treated with Proteinase K, extracted, precipitated, washed, and loaded into a 5% denaturing polyacrylamide gel. The resulting resolved bands are separated on the gel according to their size, and imaged by autoradiography, beta-scanning, or phospor-imaging systems for radiolabeled probes. Non-radioactive probes resolved on denaturing polyacrylamide gels are then transferred to a positively-charged nylon membrane. The membrane is probed with Streptavidin-HRP and enchased chemiluminescent substrate provided in BD Riboquant Non-rad detection kit (Cat. No. 551918). For non-radioactive detection, the membrane is exposed to x-ray film, and the level of each mRNA species in the original RNA sample is determined based on the intensity of the appropriately-sized, protected probe fragment.

For detailed and complete protocol visit
**www.bdbiosciences.com/pdfs/manuals/01-81014-20A.pdf**

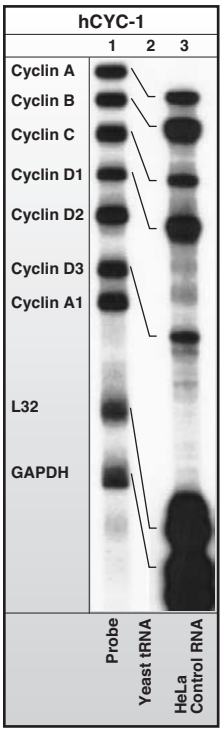



***Figure 52.*** *Samples of total RNA from various sources were analyzed for distinct mRNA species by using BD RiboQuant™ Multi-Probe Ribonuclease Protection Assay System with the hCYC-1 Multi-Probe Template Set. The autoradiogram from this analysis shows the hCYC-1 probe set not treated with RNases (Lane 1). Also shown are the corresponding RNase-protected probes following hybridization with yeast tRNA (2 µg, Lane 2) and RNA from HeLa cells (5 µg, Lane 3). Note that each probe band (Lane 1) migrates slower than its protected band (Lane 3); this is due to flanking sequences in the probe that are not protected by mRNA.*

## BD RiboQuant™ Multi-Probe Ribonuclease Protection Assay (RPA) System

| DESCRIPTION | APPS | SIZE | CAT. NO. |
|---|---|---|---|
| **BD RiboQuant RPA Starter Package** | RPA | 1 Kit | **556144** |
| *contains:* | | | |
| *In Vitro* Transcription Kit | | 1 Kit | **556850** |
| RPA Kit | | 1 Kit | **556134** |
| Choice of one BD RiboQuant Template Set | | 10 transcriptions | |
| **BD RiboQuant Non-Rad RPA Starter Package** | RPA | 1 Kit | **551919** |
| *contains:* | | | |
| BD RiboQuant Non-Rad *In Vitro* Transcription Kit | | 1 Kit | **551917** |
| RPA Kit | | 1 Kit | **556134** |
| BD RiboQuant Non-Rad Detection Kit | | 1 Kit | **551918** |
| Choice of one BD RiboQuant Template Set | | 10 transcriptions | |
| **BD RiboQuant *In Vitro* Transcription Kit** | RPA | 25 transcriptions | **556850** |
| *contains:* | | | |
| Ammonium Acetate | | 1.3 ml | **556133** |
| DTT | | 50 µl | **556126** |
| EDTA | | 650 µl | **556132** |
| GTP/ATP/CTP/UTP Pool | | 25 µl | **556125** |
| RNase-Free DNase | | 50 µl | **556130** |
| RNAsin®* | | 25 µl | **556128** |
| T7 RNA Polymerase | | 25 µl | **556129** |
| Transcription Buffer | | 100 µl | **556127** |
| Yeast tRNA | | 50 µl | **556131** |
| **BD RiboQuant Non-Rad *In Vitro* Transcription Kit** | RPA | 1 Kit | **551917** |
| *contains:* | | | |
| 5x Nucleotide Mix | | 16.5 µl | |
| 5x Transcription Buffer | | 20 µl | |
| DTT | | 10 µl | |
| Enzyme Mix (RNasin & T7 RNA Polymerase) | | 10 µl | |
| RNase-free DNase I | | 10 µl | |
| EDTA | | 135 µl | |
| Glycogen | | 5 µl | |
| LiCl | | 2.7 ml | |
| **RPA Kit** | RPA | 200 RPA reactions | **556134** |
| *contains:* | | | |
| Ammonium Acetate | | 24 ml | **556141** |
| Hybridization Buffer | | 3.6 ml | **556135** |
| Loading Buffer | | 1.3 ml | **556142** |
| Proteinase K | | 300 µl | **556139** |
| Proteinase K Buffer | | 3.9 ml | **556138** |
| RNase A + T1 Mix | | 60 µl | **556137** |
| RNase Buffer | | 25 ml | **556136** |
| Yeast tRNA | | 300 µl | **556140** |

# RNA Detection

## BD RiboQuant™ Multi-Probe Ribonuclease Protection Assay (RPA) System *(continued)*

| DESCRIPTION | APPS | SIZE | CAT. NO. |
|---|---|---|---|
| **BD RiboQuant Non-Rad Detection Kit** | RPA | 1 Kit | **551918** |
| *contains:* | | | |
| Nylon Membrane | | 10 Membranes | |
| Membrane Blocking Buffer | | 500 ml | |
| Wash Buffer (4x) | | 500 ml | |
| Substrate Equilibration Buffer | | 500 ml | |
| Streptavidin-Horseradish Peroxidase | | 1.5 ml | |
| Stable Peroxide Buffer | | 75 ml | |
| Luminol / Enhancer | | 75 ml | |

## RNA Isolation

| DESCRIPTION | APPS | SIZE | CAT. NO. |
|---|---|---|---|
| **Total RNA Isolation Kit** | RPA | 1 Kit | **556224** |

## Human Apoptosis

| Template Set | hAPO-1b | hAPO-1c | hAPO-2b | hAPO-2c | hAPO-3 | hAPO-3b |
|---|---|---|---|---|---|---|
| Catalog Number | **556209** | **556233** | **556240** | **556235** | **556163** | **556237** |
| Templates | Caspase-8 | Caspase-8 | bcl-w | bcl-w | Caspase-8 | Caspase-8 |
| | Granzyme B | Caspase-4 | bcl-x (L) | bcl-x (L) | FasL | FasL |
| | Caspase-3 | Caspase-3 | bcl-x (S) | bcl-x (S) | Fas | Fas |
| | Caspase-6 | Caspase-6 | bfl-1 | bfl-1 | FADD | CLARP |
| | Caspase-5 | Caspase-10a | BID | bad | DR3 | FAP |
| | Caspase-2 (S) | Caspase-5 | bik | bik | FAP | CRADD |
| | Caspase-7 | Caspase-2 (S) | bak | bak | FAF | DAXX |
| | Caspase-1 | Caspase-7 | bax | bax | TRAIL | MADD |
| | Caspase-2 (L) | Caspase-1 | bcl-2 | bcl-2 | TNFRp55 | RIP |
| | Caspase-9 | Caspase-2 (L) | mcl-1 | mcl-1 | TRADD | L32 |
| | L32 | Caspase-9 | L32 | L32 | RIP | GAPDH |
| | GAPDH | L32 | GAPDH | GAPDH | L32 | |
| | | GAPDH | | | GAPDH | |

| Template Set | hAPO-3d | hAPO-4 | hAPO-5 | hAPO-5b | hAPO-5c | hAPO-6 |
|---|---|---|---|---|---|---|
| Catalog Number | **557278** | **556164** | **556165** | **556236** | **556239** | **556238** |
| Templates | Caspase-8 | Granzyme A | XIAP | TRAF1 | XIAP | IPL |
| | FasL | Granzyme B | TRAF1 | TRAF2 | Survivin | ASK1 |
| | Fas | DAD1 | TRAF2 | TRAF4 | NAIP | Harakiri |
| | DcR-1 | FAST K | TRAF4 | I-TRAF | c-IAP-2 | SIAH |
| | DR3 | Granzyme H | NAIP | TRAF5 | c-IAP-1 | DFF |
| | DR5 | RVP1 | c-IAP-2 | TRAF6 | TRPM-2 | Nip2 |
| | DR4 | Dr-nm23 | c-IAP-1 | TRAF3 | L32 | Nip3 |
| | TRAIL | Granzyme 3 | TRPM-2 | TRIP | GAPDH | Nip1 |
| | DcR-2 | Requiem | TRAF3 | L32 | | DAP-K |
| | TNFRp55 | CAS | L32 | GAPDH | | DAP |
| | TRADD | Perforin | GAPDH | | | DRM |
| | RIP | L32 | | | | L32 |
| | L32 | GAPDH | | | | GAPDH |
| | GAPDH | | | | | |

## Mouse and Rat Apoptosis

| Template Set | mAPO-1 | mAPO-2 | mAPO-3 | rAPO-1 |
|---|---|---|---|---|
| Catalog Number | **556195** | **556191** | **556192** | **556227** |
| Templates | Caspase-8 | bcl-w | Caspase-8 | Fas |
| | Caspase-3 | bfl1 | FasL | bcl-x (L) |
| | Caspase-6 | bcl-x(L) | Fas | bcl-x (S) |
| | Caspase-11 | bcl-x(S) | FADD | FasL |
| | Caspase-12 | bak | FAP | Caspase-1 |
| | Caspase-2 (L) | bax | FAF | Caspase-3 |
| | Caspase-7 | bcl2 | TRAIL | Caspase-2 |
| | Caspase-1 | bad | TNFRp55 | bax |
| | Caspase-14 | L32 | TRADD | bcl-2 |
| | Caspase-2 (S) | GAPDH | RIP | L32 |
| | L32 | | L32 | GAPDH |
| | GAPDH | | GAPDH | |

## Human Cell Cycle Regulators

| Template Set | hCC-1 | hCC-2 | hCYC-1 | hCYC-2 | hStress-1 | hTS-1 |
|---|---|---|---|---|---|---|
| Catalog Number | **556159** | **556160** | **556189** | **556190** | **556188** | **556161** |
| Templates | Cdk1 | p130 | Cyclin A | Cyclin E | bcl-x | p130 |
| | Cdk2 | Rb | Cyclin B | Cyclin F | p53 | Rb |
| | Cdk3 | p107 | Cyclin C | Cyclin G1 | GADD45 | p107 |
| | Cdk4 | p53 | Cyclin D1 | Cyclin G2 | c-fos | DP1 |
| | p27 | p57 | Cyclin D2 | Cyclin I | p21 | DP2 |
| | p21 | p27 | Cyclin D3 | Cyclin H | bax | E2F1 |
| | PISSLRE | p21 | Cyclin A1 | L32 | bcl-2 | E2F2 |
| | p16 | p19 | L32 | GAPDH | mcl-1 | E2F4 |
| | L32 | p18 | GAPDH | | L32 | L32 |
| | GAPDH | p16 | | | GAPDH | GAPDH |
| | | p14/15 | | | | |
| | | L32 | | | | |
| | | GAPDH | | | | |

## Mouse Cell Cycle Regulators

| Template Set | mCC-1 | mCYC-1 | mCYC-2 |
|---|---|---|---|
| Catalog Number | **559540** | **556241** | **556242** |
| Templates | cdk1 | Cyclin A2 | Cyclin E |
| | cdk2 | Cyclin B1 | Cyclin F |
| | cdk4 | Cyclin C | Cyclin G1 |
| | cdk5 | Cyclin D1 | Cyclin G2 |
| | cdk7 | Cyclin D2 | Cyclin I |
| | cdk8 | Cyclin D3 | Cyclin H |
| | KKIALRE | Cyclin A1 | L32 |
| | PCTAIRE3 | Cyclin B2 | GAPDH |
| | PCTAIRE1 | L32 | |
| | PITAIRE/CHED | GAPDH | |
| | PITALRE | | |
| | PITSLRE | | |
| | L32 | | |
| | GAPDH | | |

# RNA Detection

## Human DNA Repair Pathways

| Template Set | hBER-1a* | hBER-2* | hDisR-1* | hDSBR-1* | hDSBR-2* | hMMR* | hNER-1* | hNER-2* | hNER-3* |
|---|---|---|---|---|---|---|---|---|---|
| Catalog Number | Custom | Custom | Custom | Custom | Custom | Custom | Custom | Custom | Custom |
| Templates | SMUG1 | POLε | APC | LIM15 | ATM | PMS1 | XPG | hCSB | POLε |
| | OGG1 | PARP | RB1 | RAD50 | NBS1 | PMS2 | DDB1 | hXPB | RFCp140 |
| | TDG | POLδ | BLM | RAD54 | XRCC4 | MSH2 | XPC | hTFIIHp52 | POLδ |
| | APEX | LIG3 | WRN | RAD52 | LIG4 | MSH3 | XPF | hTFIIHp44 | LIG1 |
| | UNG | LIG1 | FANCC | MRE11 | XRCC2 | MSH6 | RPAp70 | hCSA | RFCp40 |
| | MPG | XRCC1 | FANCA | XRCC2 | XRCC3 | MSH5 | DDB2 | hcdk7 | RFCp38 |
| | NTHL1 | POLβ | BRCA1 | XRCC3 | XRCC9 | MYH | HHR23B | hCyclinH | RFCp37 |
| | RPA4 | FEN1 | BRCA2 | RAD51 | Ku86 (XRCC5) | MLH1 | XPA | hTFIIHp34 | RFCp36 |
| | MGMT | PCNA | WT1 | RAD51B | DNA-PK (XRCC7) | L32 | RPAp32 | ERcc1 | PCNA |
| | MBD4 | L32 | L32 | RAD51C | Ku70 (XRCC6) | GAPDH | RPAp14 | XPD | L32 |
| | L32 | GAPDH | GAPDH | RAD51D | L32 | | L32 | L32 | GAPDH |
| | GAPDH | | | L32 | GAPDH | | GAPDH | GAPDH | |
| | | | | GAPDH | | | | | |

*Only available as a Custom Template Set. Please contact our Technical Services Team at 877.232.8995 option 2.1 or email techserv@bd.com for more information.

## Mouse and Rat DNA Repair Pathways

| Template Set | mBER-1* | mBER-2* | rBER-1* |
|---|---|---|---|
| Catalog Number | Custom | Custom | Custom |
| Templates | OGG1 | PARP | OGG1 |
| | TDG | POLδ | PARP |
| | APEX | LIG3 | APE |
| | UNG | LIG1 | POLδ |
| | MPG | XRCC1 | MPG |
| | NTHL1 | FEN1 | POLβ |
| | MGMT | POLβ | MGMT |
| | L32 | PCNA | PCNA |
| | GAPDH | L32 | L32 |
| | | GAPDH | GAPDH |

*Only available as a Custom Template Set. Please contact our Technical Services Team at 877.232.8995 option 2.1 or email techserv@bd.com for more information.

## Human Toxicology

| Template Set | hTox-1b | hTox-2 | hTox-3 | hTox-4 |
|---|---|---|---|---|
| Catalog Number | 550793 | 550794 | 552134 | 552135 |
| Templates | CYP2B6 | FMO1 | UGT1A1 | GSTM3 |
| | NCPR | FMO2 | UGT1A7 | GSTM2 |
| | CYP2C8 | FMO3 | UGT1A10 | GSTM1 |
| | CYP1A2 | FMO4 | UGT1A9 | GSTM4 |
| | CYP2A6 | FMO5 | UGT1A6 | GSTM5 |
| | CYP2C9 | SULT2A1 | UGT1A8 | GSTA4 |
| | CYP2C19 | SULT2B1 | UGT1A4 | GSTA2 |
| | CYP3A4 | SULT1C1 | UGT2B4 | GSTA3 |
| | CYP2D6 | SULT1A | UGT2B15 | L32 |
| | L32 | L32 | UGT2B7 | GAPDH |
| | GAPDH | GAPDH | L32 | |
| | | | GAPD | |

# Notes

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