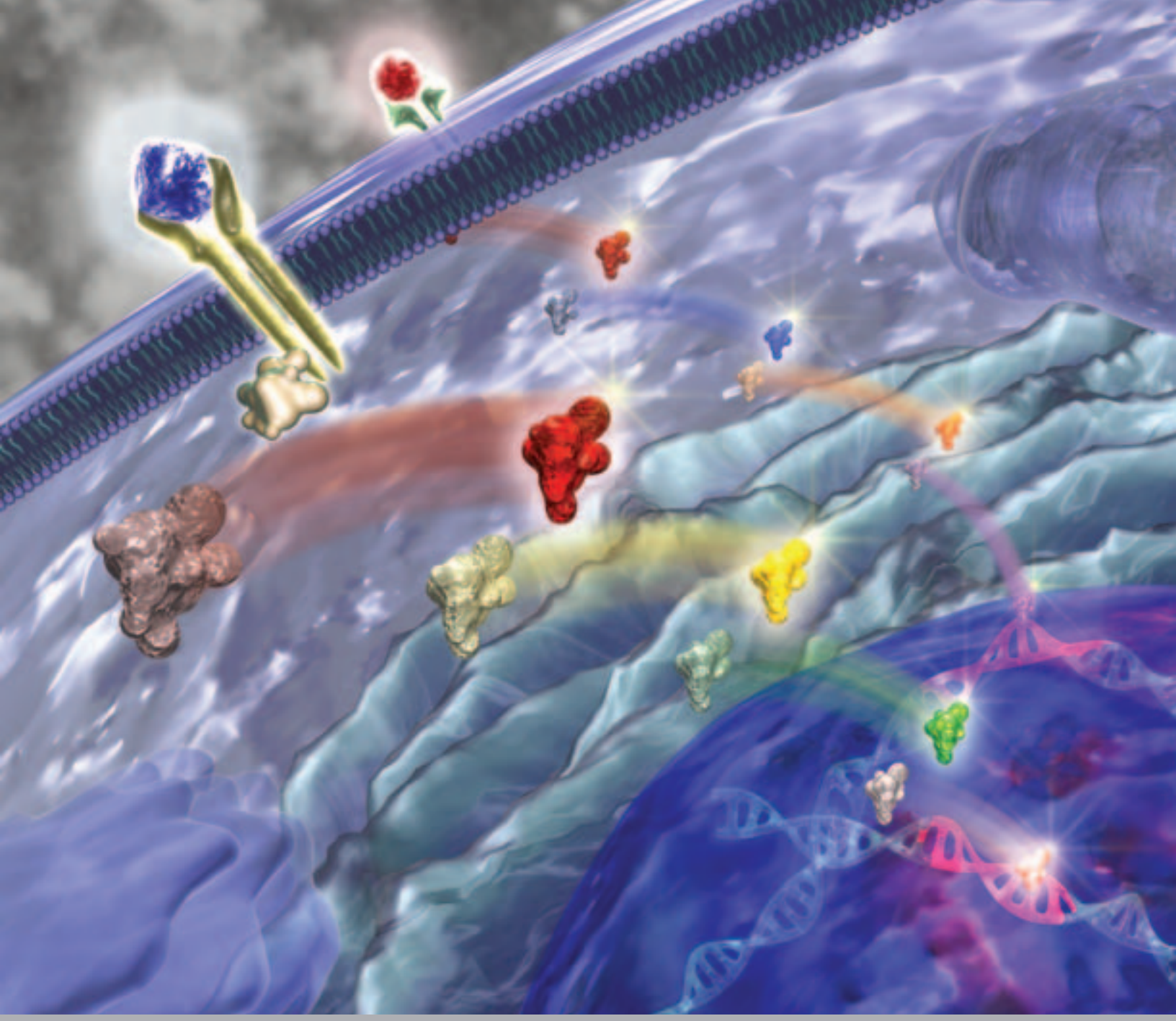


Phospho-Protein Profiling

BD

Table of Contents

Prices are subject to change.
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.

Introduction to Phosphorylation-State Specific Protein Profiling

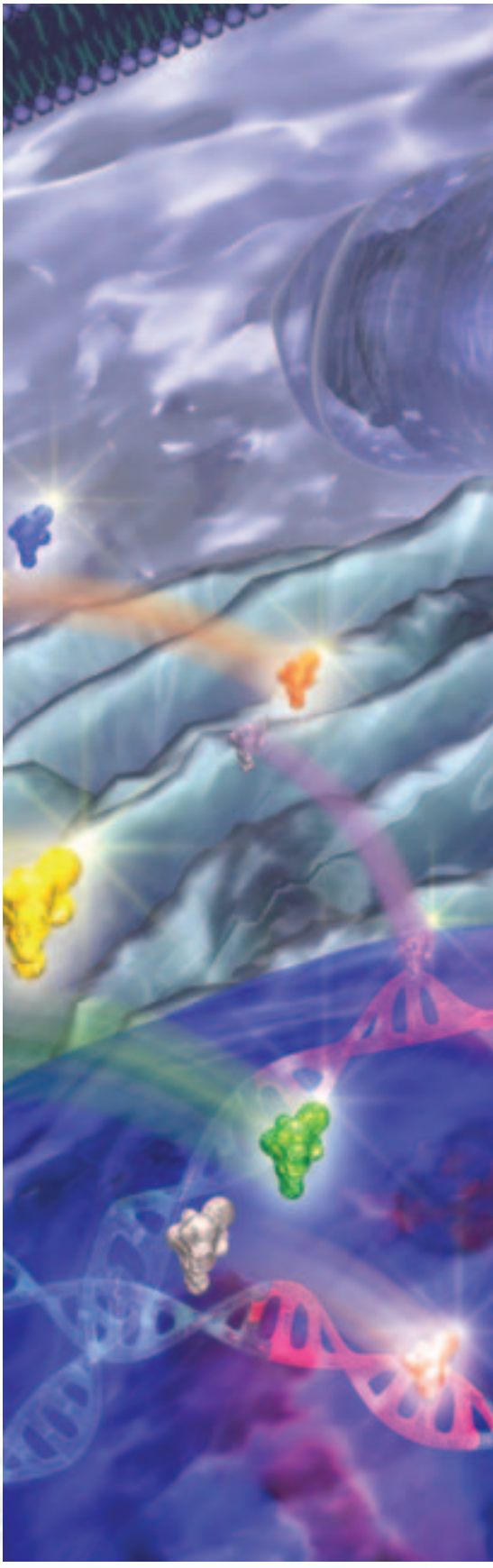
Phosphorylation of tyrosine, serine, and threonine residues is critical for the control of protein activity involved in various cellular events. An assortment of kinases and phosphatases regulate intracellular protein phosphorylation in many different cell signaling pathways, such as T and B cell signaling, those regulating apoptosis, growth and cell cycle control, plus those involved with cytokine, chemokine, and stress responses.

Historically, phosphoprotein detection has been performed using techniques such as radiometric kinase assays and phosphoamino acid labeling. However, the advent of phospho-specific antibodies has facilitated the use of more straightforward techniques such as Western blotting, immunoprecipitation, and immunofluorescence microscopy.

These techniques, however, have several shortcomings in that they require a relatively large amount of sample, are time-consuming, do not produce truly quantitative results, and are not conducive to multiparameter analysis. Emerging technology from Garry Nolan, PhD at Stanford University has revolutionized historical approaches to phosphoprotein analysis. This technology combines phospho-specific antibodies with the power of flow cytometry to enhance phosphoprotein study in ways not before possible.

Flow cytometry requires only a small sample size and is ideal for performing rapid, quantitative, multiparameter analyses of single cells and distinct cell subpopulations. As a worldwide leader in flow cytometry reagents and analysis, BD Biosciences has joined Dr. Nolan in a collaborative effort to make the technology and reagents available to the research community. Thus, we have launched BD™ Phosflow reagents to enable intracellular phosphoprotein analysis of single cells or subpopulations using flow cytometry. Furthermore, we have extended this approach by employing flow cytometry using the BD™ Cytometric Bead Array to analyze protein phosphorylation in cell and tissue lysates.

BD Biosciences offers a variety of antibodies for the detection of phosphorylation motifs and produces quality antibodies that will meet and exceed your research needs, whether your experiment calls for Western blotting, immunoprecipitation, immunofluorescence microscopy, or flow cytometry. On the pages that follow, you'll find descriptions and sample data that illustrate the power and versatility of our high-quality phospho-specific antibodies.



BD™ Phosflow Multiplexed Phosphorylation-State Analysis in Single Cells

Until recently, the study of intracellular signaling pathways was limited by the inflexibility of available techniques. Traditional methods like Western blot, immunoprecipitation, and immunofluorescence microscopy, did not allow researchers to correlate intracellular signaling events with distinct subpopulations of cells.

By offering multi-channel, multiparameter control, flow cytometry is proving to be a flexible and powerful addition to these techniques. Not only is it rapid, sensitive, and quantitative, but it enables the differential evaluation of intracellular signaling events directly in complex primary samples such as whole blood or peripheral blood mononuclear cells. Particularly for immune cell function analysis, it is necessary to study intracellular signaling pathways in their most native context.

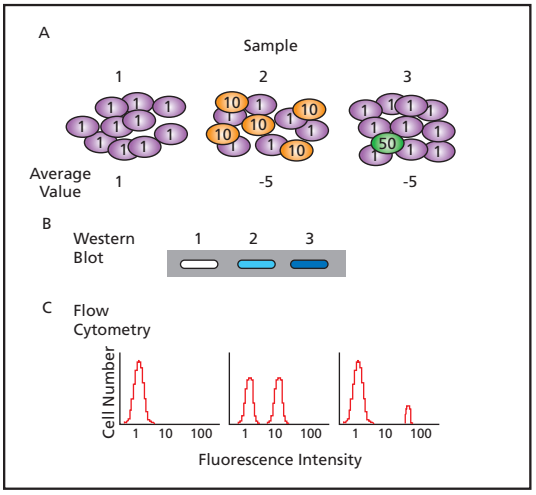


Figure 1. *A theoretical experiment comparing Western blot and flow cytometry with three samples and a protein of interest at 1, 10, or 50 copies per cell. Sample 2 and 3 look the same via Western blot, but when stained with fluorescently labeled antibodies, the differences between the samples become more relevant. (Source: P.O. Krutzik et al. Clinical Immunology 110 (2004) 206–221)*

BD™ Phosflow enables measurement of activation-states of multiple proteins on a single-cell level. Combining surface marker immunophenotyping and intracellular signal assessment, it is possible to obtain mechanistic and kinetic information of subset specific signaling.

By choosing BD Phosflow reagents you'll get high quality antibodies conjugated to a variety of fluorophores facilitating three-, four-, five, and six-color experiments.

Kinetic and mechanistic understanding of cellular responses based on kinase activity would be beneficial to many fields of immunology since fine subset analysis is not possible by conventional techniques. Furthermore, the complexity of cellular subsets that exist within the largely attributed groups of T cells, B cells, NK cells, and monocytes illustrates that subsets can differentially function in immune responses as is the case with naïve and memory T cells. Thus, it is important to study intracellular signaling mechanisms both in isolation and within the context of other contacting cells.

Western blot	Flow cytometry
Population analysis Obtain average value of multiple cells	**Single cell analysis** Collect data for each individual cell
Homogeneous sample Limited to cultured or purified cells	**Heterogeneous cell types** Complex primary samples, such as immune cells
One parameter Obtain data sets individually	**Multiparameter** Correlate multiple markers simultaneously
Large number of cells Requires *in vitro* derived cultures	**Small number rare cell subsets** Direct analysis of rare cell types
Time consuming for large sample sets Not amenable to large screening efforts	**Rapid and scalable** Performed in 96-well plates and in parallel
Protein size and antibody specificity Antibody selectivity for target is clearly visible	**Antibody must be validated** Antibody must have high affinity and selectivity

Table 1. *Comparison of phospho-specific flow cytometry and traditional Western blotting (Source: P.O. Krutzik et al. Clinical Immunology 110 (2004) 206–221)*

For your convenience, BD Biosciences now has validated directly labeled conjugates for multiparameter analysis of phosphorylation events in T and B Cells directly in human whole blood and PBMCs.

PerCP-CY5.5	PE	Alexa Fluor® 488	Alexa Fluor® 647
CD3	CD3	Lck*	p38
CD20	CD4	p38	p44 (ERK1/2)
	CD8	p44 (ERK1/2)	stat1
	Lck	stat1	stat3
	p38	stat3	stat5
	p44 (ERK1/2)	stat5	Zap70
	stat1	stat6	
	stat3	Zap70	
	stat5		
	stat6		
	Zap70		

** has not yet been optimized for whole blood*

Table 2. Validated reagents for multiplexed analysis of T- and B-Cells in PBMCs. *All possible combinations shown in Table 2 have been validated in whole blood and PBMC samples. Please visit* **www.bdphosflow.com** *for sample data, recommended color combinations and protocols.*

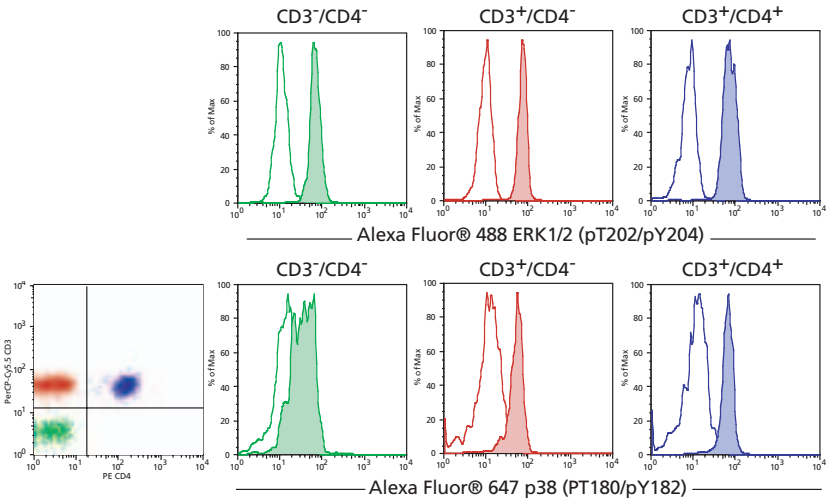


Figure 2. Multicolor analysis of human PBMCs stained with PerCP-Cy5.5 anti-human CD3, PE anti-human CD4, Alexa Fluor® 647 anti-p38 (pT180/pY182), and Alexa Fluor® 488 anti-ERK1/2 (pT202/pY204). *Human PBMCs were either left untreated (open histogram) or treated with PMA (shaded histogram and CD3, CD4 dot blot), 40 nM for 15 minutes at 37°C. The cells were then fixed using BD Cytofix™ Buffer for 10 minutes at 37°C, and followed by BD™ Phosflow Perm Buffer III for 30 minutes on ice.*

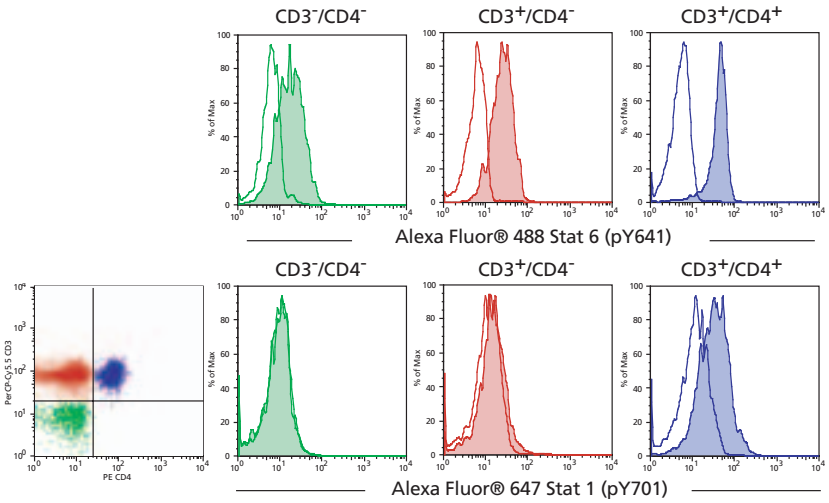


Figure 3. Multicolor analysis of human PBMCs stained with PerCP-Cy5.5 anti-human CD3, PE anti-human CD4, Alexa Fluor® 488 anti-Stat 6 (pY641), and Alexa Fluor® 647 anti-Stat 1 (pY701). *Human PBMCs were either left untreated (open histogram) or treated with IL-6 + IL-4 (shaded histogram and CD3, CD4 dot blot), 100 ng/ml each for 15 minutes at 37°C. The cells were then fixed using BD Cytofix™ Buffer for 10 minutes at 37°C, and followed by BD™ Phosflow Perm Buffer III for 30 minutes on ice.*

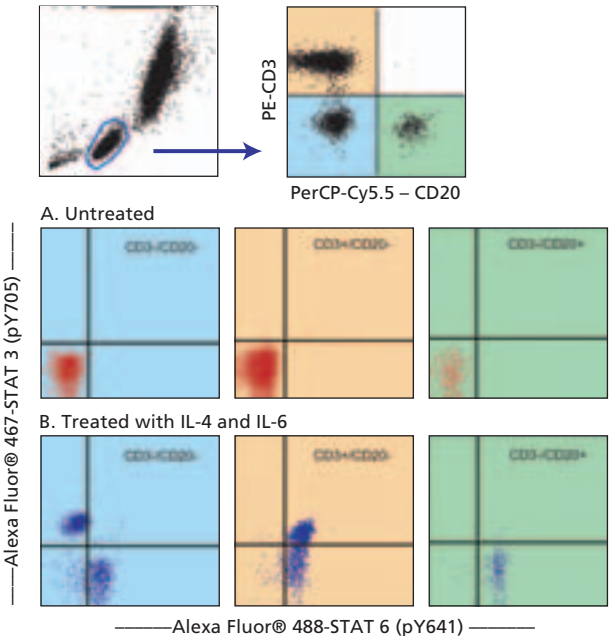


Figure 4. Our patented BD™ Phosflow technology enables phosphorylation-state analysis directly in human whole blood. After stimulation, our proprietary BD Phosflow Lyse/Fix buffer lyses red and fixes white blood cells. *Human whole blood was either untreated (A) or treated with IL-6 + IL-4 (B), 100 ng/ml each for 15 minutes at 37°C. The cells were then fixed using BD™ Phosflow Lyse/Fix Buffer for 10 minutes at 37°C, and followed by Perm Buffer III for 30 minutes on ice.*

BD™ Phosflow for Whole Blood or PBMC Samples *(continued)*

BD™ Phosflow Reagents

DESCRIPTION	REACT	CLONE	ISOTYPE	FORMAT	SIZE	CAT. NO.
AKT (pT308)	Hu	J1-223.371	Mouse IgG_1, κ	PE	50 tests	**558275**
AKT (pS473)	Hu	F29-763	Rabbit IgG	Alexa Fluor® 647	50 tests	**558314**
Btk (pY551) & Itk (pY511)	Hu	24a/BTK (Y551)	Ms IgG_1	PE	50 tests	**558129**
				Alexa Fluor® 488	50 tests	**558130**
				Alexa Fluor® 647	50 tests	**558134**
c-Cbl (pY700)	Hu	47/c-Cbl (Y700)	Ms IgG_1	PE	50 tests	**558087**
				Alexa Fluor® 488	50 tests	**558101**
				Alexa Fluor® 647	50 tests	**558100**
c-Cbl (pY774)	Hu	29/c-Cbl (Y774)	Ms IgG_1	PE	50 tests	**558102**
				Alexa Fluor® 647	50 tests	**558103**
Caveolin-1 (pY14)	Hu, Ms, Rat	56	Ms IgG_1	PE	50 tests	**612568**
ERK1/2 (pT202/pY204)	Hu, Ms, Rat	20a	Ms IgG_1	PE	50 tests	**612566**
				Alexa Fluor® 488	50 tests	**612592**
				Alexa Fluor® 647	50 tests	**612593**
Histone H3 (pS28)	Hu, Ms	HTA28	Rat IgG_{2a}, κ	Alexa Fluor® 647	50 tests	**558217**
JNK (PT183/PY185)	Hu	Poly	Rabbit IgG	Purified	50 tests	**558268**
MEK 1/2 (pS222)	Hu	Poly	Rabbit IgG	Purified	50 tests	**558281**
p38 MAPK (pT180/pY182)	Hu, Ms, Rat	36	Ms IgG_1	PE	50 tests	**612565**
				Alexa Fluor® 488	50 tests	**612594**
				Alexa Fluor® 647	50 tests	**612595**
PDGFRb (pY1009)	Hu	J25-602	Rat IgG_{2b}, κ	PE	50 tests	**558322**
			Rat IgG_{2b}, κ	Alexa Fluor® 647	50 tests	**558323**
PLCγ1 (pY783)	Hu	27	Ms IgG_1	Alexa Fluor® 488	50 tests	**557884**
				Alexa Fluor® 647	50 tests	**557883**
Rb (a.a. 332-344)	Hu, Ms, Rat, Monkey	G3-245, MOPC-21	Ms IgG_1	FITC Set	50 tests	**556538**
				PE Set	50 tests	**556539**
Rb, underphosphorylated (a.a. 514-610)	Hu	G99-549, MOPC-21	Ms IgG_1	FITC Set	50 tests	**550501**
				PE Set	50 tests	**550502**
Stat1 (pY701)	Hu, Ms	4a	Ms IgG_{2a}	PE	50 tests	**612564**
				Alexa Fluor® 488	50 tests	**612596**
				Alexa Fluor® 647	50 tests	**612597**
Stat3 (pS727)	Hu	49/p-Stat3	Ms IgG_1	Alexa Fluor® 488	50 tests	**558085**
				Alexa Fluor® 647	50 tests	**558099**
Stat3 (pY705)	Hu, Ms	4	Ms IgG_{2a}	PE	50 tests	**612569**
				Alexa Fluor® 488	50 tests	**557814**
				Alexa Fluor® 647	50 tests	**557815**
Stat4 (pY693)	Hu	38/p-Stat4	Ms IgG_{2b}	PE	50 tests	**558249**
				Alexa Fluor® 488	50 tests	**558136**
				Alexa Fluor® 647	50 tests	**558137**
Stat5 (pY694)	Hu	47	Ms IgG_1	PE	50 tests	**612567**
				Alexa Fluor® 488	50 tests	**612598**
				Alexa Fluor® 647	50 tests	**612599**
Stat6 (pY641)	Hu	18	Ms IgG_{2a}	PE	50 tests	**612701**
				Alexa Fluor® 488	50 tests	**612600**
				Alexa Fluor® 647	50 tests	**612601**
Stat6 (pY641)	Ms	J71-773.58.11	Ms IgG1, k	PE	50 tests	**558252**
				Alexa Fluor® 488	50 tests	**558243**
				Alexa Fluor® 647	50 tests	**558242**
ZAP70 (pY319)/SYK (pY352)	Hu	17a	Ms IgG_1	PE	50 tests	**557881**
				Alexa Fluor® 488	50 tests	**557818**
				Alexa Fluor® 647	50 tests	**557817**

Supporting Reagents

DESCRIPTION	REACT	CLONE	ISOTYPE	FORMAT	SIZE	CAT. NO.
BD Cytofix Fixation Buffer					100 mls	**554655**
BD Phosflow Fix Buffer					250 ml	**557870**
BD Phosflow Lyse/Fix Buffer (5X)					250 ml	**558049**
BD Phosflow Perm/Wash Buffer I					125 ml	**557885**
BD Phosflow Perm Buffer II					125 ml	**558052**
BD Phosflow Perm Buffer III					125 ml	**558050**
Staining Buffer (FBS)					500 mls	**554656**
Mouse IgG_1 Isotype Control		MOPC-21	Ms IgG_1	Alexa Fluor® 488	50 tests	**557782**
				Alexa Fluor® 647	50 tests	**557783**
				PE	50 tests	**551436**
Mouse IgG_{2a} Isotype Control		MOPC-173	Ms IgG_{2a}	PerCP-Cy5.5	50 tests	**558020**
CD3	Hu	SK7	Ms IgG_1	PerCP-Cy5.5	50 tests	**340949**
CD3	Hu	UCHT1	Ms IgG_1	PE	100 tests	**555333**
CD4	Hu	RPA-T4	Ms IgG_1	PE	100 tests	**555347**
CD8	Hu	RPA-T8	Ms IgG_1	PE	100 tests	**555367**
CD20 (cytoplasmic)	Hu	H1	Ms IgG_{2a}	PerCP-Cy5.5	50 tests	**558021**

Flow Cytometry Instrumentation

DESCRIPTION	CAT. NO.
BD FACSArray bioanalyzer*	inquire
• 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*

For more information on BD Biosciences instruments see **bdbiosciences.com/immunocytometry_systems**

BD™ Cytometric Bead Array (CBA) for Multiplexed Quantification of Phospho-Proteins

The BD™ Cytometric Bead Array (CBA) uses a series of particles with discrete fluorescent intensities to simultaneously detect multiple analytes from a single cell lysate. The specific capture beads are mixed with the phycoerythrin-conjugated detection antibodies and then incubated with recombinant protein standards or test samples to form sandwich complexes. Following acquisition of sample data using the flow cytometer, the sample results are generated in graphical and tabular format using the BD™ CBA Analysis Software. Multiple specificities can be analyzed simultaneously from the same sample in a flexible format. The assays can be run on any BD dual-laser cytometer. However, the BD FACSArray™, which is a plate based system, is particularly well suited to this task.

Unlike conventional assays used to detect or measure phosphorylated proteins like immunoprecipitation and Western blotting, the BD™ CBA Kits can provide sensitive, quantitative measurements of protein phosphorylation in considerably less time (approximately 5 hours). In addition, the phosphorylation status of multiple proteins can be measured on the same sample in a quantitative fashion. A list of the kits available can be seen in the product list on ***page 10***. The assays are designed to measure either phosphorylated or total specificities from a cell lysate. The specificity of the assays has been validated by immunoprecipitation and Western blotting to ensure that only the protein of interest is detected. Test samples are run against a standard curve which is generated using dilutions of a phosphorylated, recombinant standard. We have assessed the linearity of the assays by making dilutions of activated cell lysates and comparing them against the standard curve. In all cases the dilution curve of the sample is parallel to the standard curve. Reproducibility assays, both intra-assay and inter-assay demonstrated a low %CV. The sensitivity of the assay is comparable to or better than a conventional Western blot.

BD Cytometric Bead Array	BD Phosflow
Phospho-Protein detection in cell lysates Uses antibody pairs in a sandwich approach to detect phospho-proteins	**Intracellular staining of phospho-proteins** Uses directly conjugated antibodies to detect phospho-proteins in fixed and permeabilized cells
Population analysis Provides an average value for multiple cells	**Single cell analysis** Provides data for each individual cell
Highly multiplexed analysis With up to 75 answers per sample	**Phenotyping and multiplexed activation state analysis** Using BD Phosflow in combination with cell surface markers in heterogeneous cell populations
Quantitative	**Semi-quantitative**
Time to results 4 hours	**Time to results** 3 hours
Sensitivity Similar to Western Blot Analysis (ECL)	**Sensitivity** Detects phosphorylation events in single cells
Typical sample Cell Lines, tissues, purified primary cells, PBMCs	**Typical Sample** Heterogeneous samples like whole blood or PBMCs, cell lines
96-well compatible Using the BD FACSArray Bioanalyzer	**96-well compatible** Using the BD FACSArray Bioanalyzer

Table 3. Comparison of BD Phosflow and BD Cytometric Bead Array

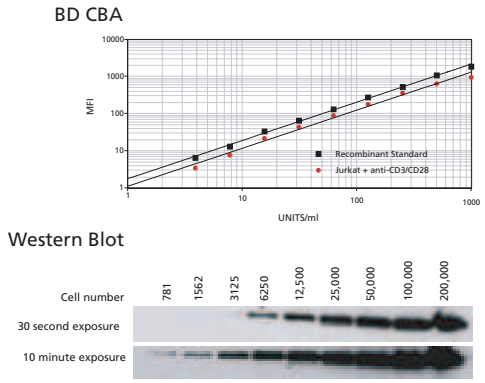


Figure 5. *Jurkat cells were activated by adding anti-CD3 and anti-CD28 which were crosslinked by anti-mouse Ig for 2 minutes. The reaction was stopped by the addition of SDS (1% final) whereupon the samples were placed in a boiling water bath. A phosphorylated recombinant standard was used to generate a standard curve. The upper panel shows the standard curve versus a titration of the activated Jurkat lysate. An important characteristic of the assay is that these two curves are parallel to each other. This insures linearity of the readings so that twice as much lysate will have twice as many units/ml. The same concentrations of lysates were also run on an SDS-PAGE gel followed by immunoblotting with an anti-phospho-ZAP70 antibody. Both a 30 second and a 10 minute exposure are shown. The BD™ CBA assay is at least as sensitive as a Western blot. In this particular example, phosphorylation of ZAP-70 can be detected in a CBA assay using lysate from less than 1000 cells.*

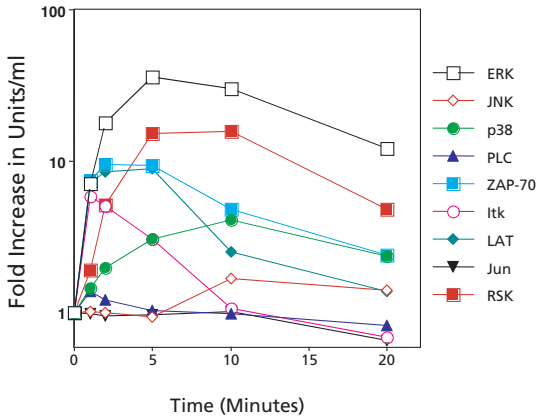
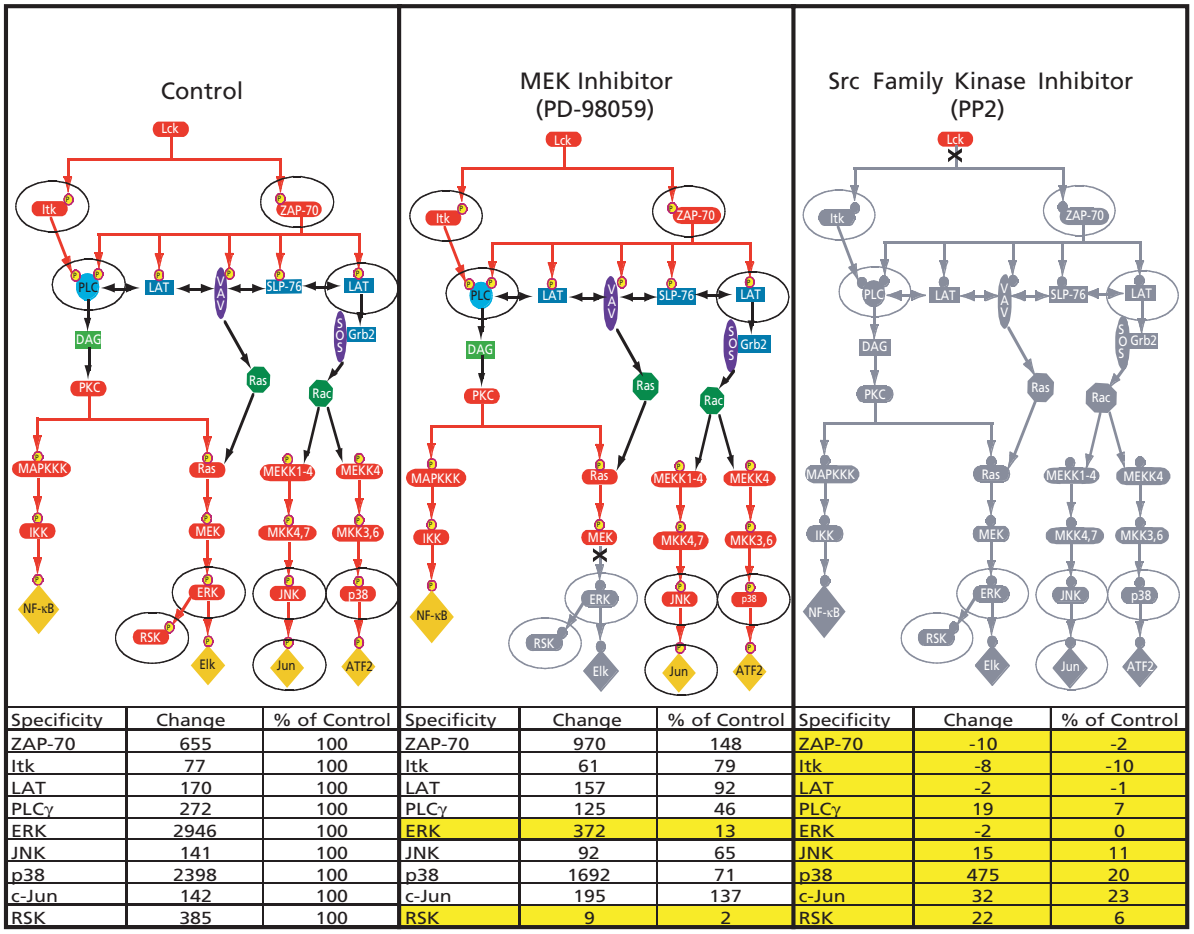


Figure 6. Kinetic analysis of T cell activation by anti-CD3/CD28. *Jurkat cells were activated with anti-CD3 and anti-CD28 for different lengths of time. Lysates were prepared as outlined in Figure 5. A 9-plex BD™ CBA Flex Set assay using 10 µg of lysate was run measuring phosphorylated ERK, JNK, p38, PLCγ, ZAP-70, Itk, LAT, c-Jun, and RSK. Using standard curves, units/ml were determined for each specificity and the fold increase in activity was plotted.*



Specificity	Change	% of Control
ZAP-70	655	100
Itk	77	100
LAT	170	100
PLCγ	272	100
ERK	2946	100
JNK	141	100
p38	2398	100
c-Jun	142	100
RSK	385	100

Specificity	Change	% of Control
ZAP-70	970	148
Itk	61	79
LAT	157	92
PLCγ	125	46
ERK	372	13
JNK	92	65
p38	1692	71
c-Jun	195	137
RSK	9	2

Specificity	Change	% of Control
ZAP-70	-10	-2
Itk	-8	-10
LAT	-2	-1
PLCγ	19	7
ERK	-2	0
JNK	15	11
p38	475	20
c-Jun	32	23
RSK	22	6

Figure 7. Effect of inhibitors on T cell signaling. *Jurkat cells were pre-incubated with either buffer, 200 µM PD-98059 (MEK inhibitor), or 10 µm PP2 (Src family tyrosine kinase inhibitor) for 20 minutes before being activated with anti-CD3/CD28 for 2 minutes. In the left panel, a T cell signaling pathway is shown. The 9 phospho-specificities that were tested simultaneously are circled. The table at the bottom of the panel shows the units/ml for each specificity. The middle panel shows the effects of pre-incubation with PD-98059. Since this compound inhibits MEK, only ERK and RSK should be affected. This is shown in gray on the pathway. However, although ERK and RSK are almost completely inhibited, this compound does have effects on other signaling molecules. In the right panel, Jurkat cells were pre-incubated with PP2 which inhibits Src family kinases such as Lck. This should shut down all of the signaling which is what is seen in the table.*

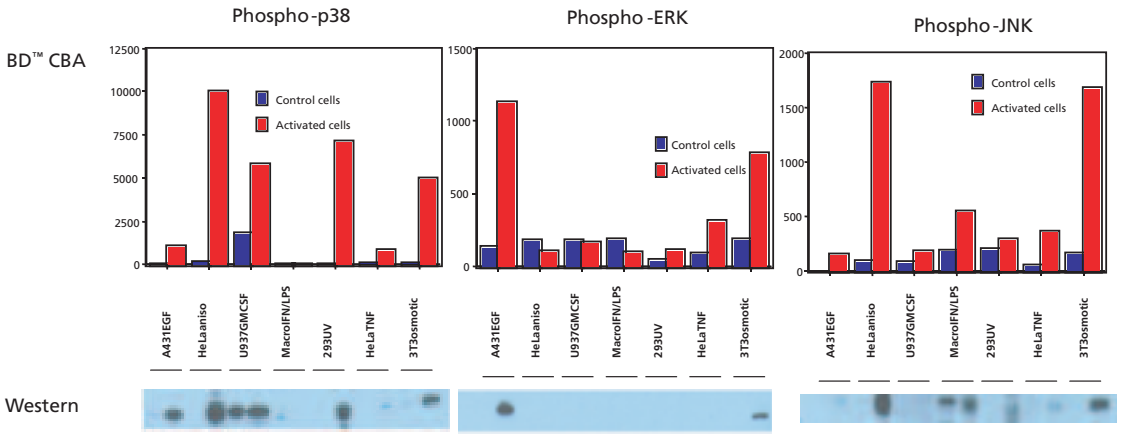


Figure 8. Activation of MAP kinases by different stimuli. *Different cell types were activated with different stimuli. Cell lysates were prepared as described in* ***Figure 1*** *and a 3-plex CBA was run to measure phosphorylated MAP kinases (ERK, JNK, p38). The bottom panel shows a Western blot of the same lysates using phosphospecific antibodies. Different stimuli have different patterns of activation and the results from the CBA assay correlate well with the results from the Western blots.*

BD FACSArray™ Bioanalyzer for BD™ CBA Applications

BD™ CBA Phospho Flex Sets Product List:

DESCRIPTION	REACT	FORMAT	SIZE	CAT. NO.
Phospho Btk (Y551) Flex Set (Bead D5)	Hu, Ms	BD CBA Flex Set	100 tests	**558236**
Phospho eNos (S1177) Flex Set (Bead C7)	Hu, Ms	BD CBA Flex Set	100 tests	**558239**
Phospho ERK1/2 (T202/Y204) Flex Set (Bead C4)	Hu, Ms	BD CBA Flex Set	100 tests	**558234**
Phospho Itk (Y511) Flex Set (Bead C6)	Hu, Ms	BD CBA Flex Set	100 tests	**558230**
Phospho JNK1/2 (T183/Y185) Flex Set (Bead B5)	Hu, Ms	BD CBA Flex Set	100 tests	**558235**
Phospho p38 (T180/Y182) Flex Set (Bead B6)	Hu, Ms	BD CBA Flex Set	100 tests	**558233**
Phospho PLC-g (Y783) Flex Set (Bead B7)	Hu, Ms	BD CBA Flex Set	100 tests	**558228**
Phospho RSK (T573) Flex Set (Bead D7)	Hu, Ms	BD CBA Flex Set	100 tests	**558240**
Phospho Stat1 (Y701) Flex Set (Bead C5)	Hu, Ms	BD CBA Flex Set	100 tests	**558222**
Phospho Syk (Y352) Flex Set (Bead B9)	Hu, Ms	BD CBA Flex Set	100 tests	**558237**
Phospho ZAP70 (Y319) Flex Set (Bead B8)	Hu, Ms	BD CBA Flex Set	100 tests	**558229**
Total STAT1 Flex Set (Bead D4)	Hu, Ms	BD CBA Flex Set	100 tests	**558227**
Total Syk Flex Set (Bead B9)	Hu, Ms	BD CBA Flex Set	100 tests	**558238**
Total ZAP70 Flex Set (Bead B8)	Hu, Ms	BD CBA Flex Set	100 tests	**558232**

Coming Soon: BLNK, c-jun, eNOS, LAT, Pyk2, RSK, SLP-76

The BD FACSArray™ bioanalyzer, the latest instrument released from BD Biosciences, 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 delivered 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 read and analyzed in less than 35 minutes, while saving 1000 events per sample well. Each event contains information of 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

Phospho-STAT Detection in Immunohistochemistry

Using Immunohistochemistry (IHC), protein phosphorylation now can also be studied *In Situ*. At BD Biosciences we have validated our phosphorylation site specific antibodies across different applications. The same monoclonal antibody that detects total phospho-protein concentration in lysates, now can also be used to study protein localization within cells and tissues. IHC allows researchers to visualize protein-phosphorylation in its micro-environment and to study protein protein interaction and translocation events simultaneously.

We have used different *In Vivo* stimulation rat models and an *Ex-Vivo* stimulation model using human tonsils to study protein activation *In Situ*. In addition, we also have looked at a variety of tumor tissues to detect the up and down regulation of phospho-specific markers. For each model, the specificity of antibodies has been validated by Western blotting. As a control, we have used phosphatase-treated tissue sections to verify phospho-specific binding. Please refer to our "Antibodies for Phospho-Protein Analysis" product list (page 12 – 20) for antibodies validated for IHC.

Stat1 Staining on Rat Liver Sections

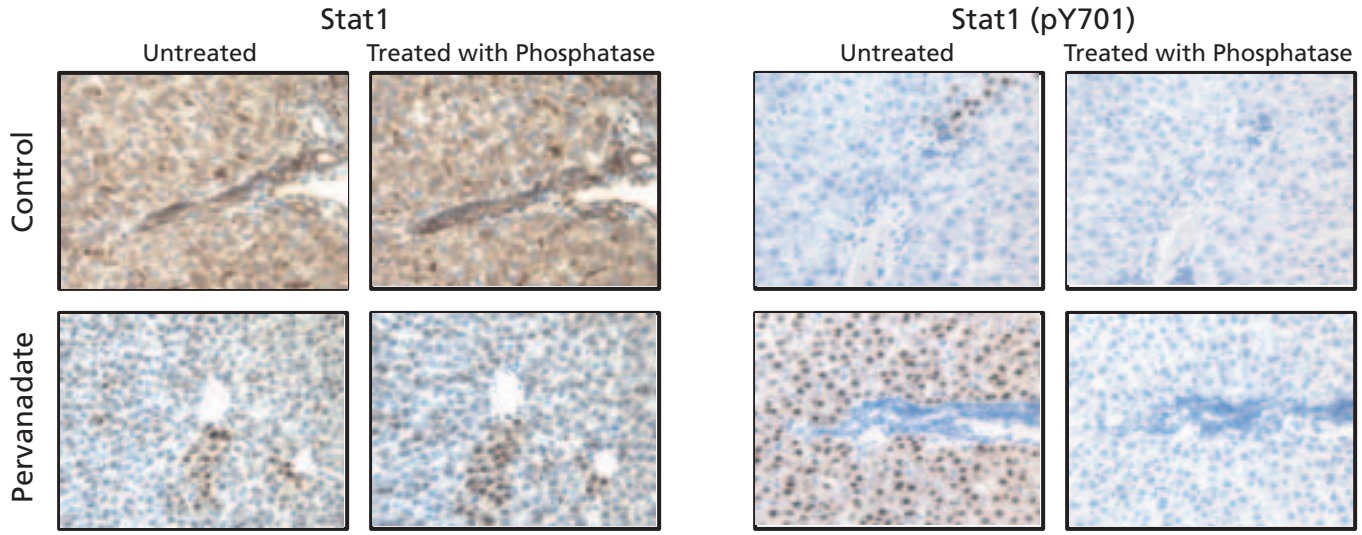


Figure 9. Stat1 staining on rat liver. *The solutions of pervanadate or PBS were injected intraperitoneally into rats at a dose of 10 µl/g of body weight. The liver was removed and fixed in formalin, processed, and sectioned. The liver sections were then either left untreated or treated with a phosphatase to eliminate all phosphorylation. The tissue sections were stained with purified Stat1 antibody (Cat. No. 610185) or purified Stat1 (pY701) (Cat. No. 612232).*

Stat6 Staining on Human Tonsil Sections

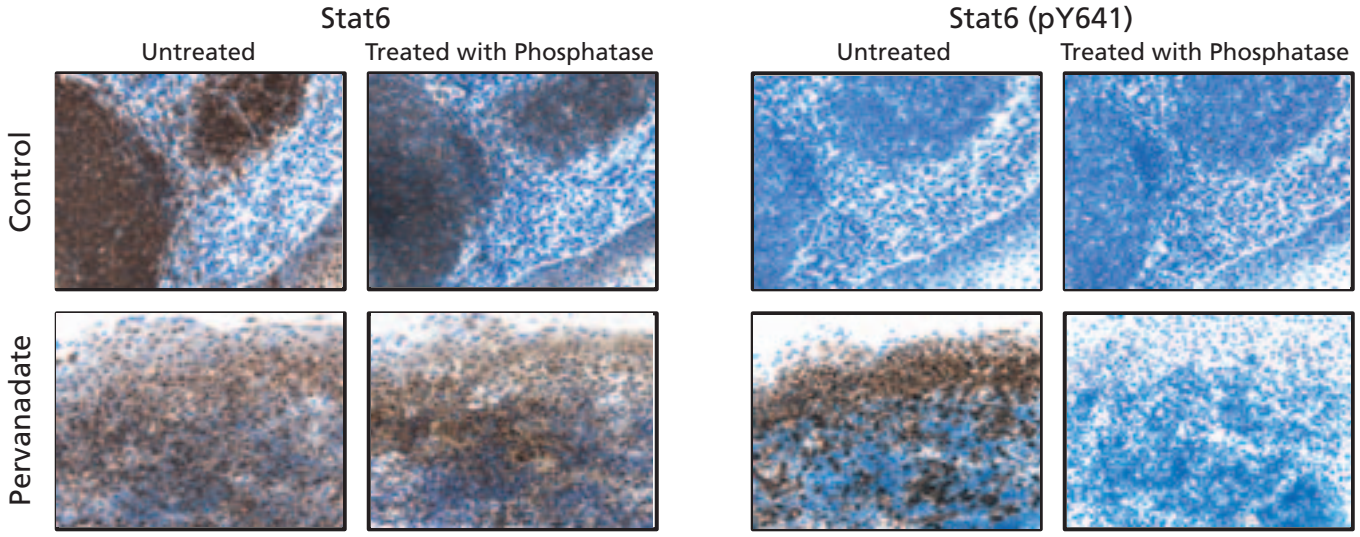


Figure 10. Stat6 staining on human tonsil. *Fresh human tonsil was either incubated in PBS (Control) or 5 mM Pervanadate solution for 2 hours. Following stimulation the tonsil was fixed in formalin and processed. The tonsil sections were then either left untreated or treated with a phosphatase to eliminate all phosphorylation. The tissue sections were stained with purified Stat6 antibody (Cat. No.611290) or purified Stat6 (pY641) (Cat. No. 611820).*

Antibodies for Phospho-Protein Analysis

Serine, Theronine, and Tyrosine Phosphorylation Detection

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
Phosphoserine	Hu, Rat	19	IF, WB	Purified	50 µg/150 µg	**612546/47**
Phosphoserine/Threonine	Hu, Rat	22a	IF, WB	Purified	50 µg/150 µg	**612548/49**
Phosphotyrosine	C, D, F, Hu, Ms, Rat	PY20	FCM, IF, IHC, IP, WB	Purified	1 mg	**610000**
Phosphotyrosine	C, D, F, Hu, Ms, Rat	PY20	IP, WB	Biotin	50 µg/150 µg	**610007/08**
Phosphotyrosine	C, D, F, Hu, Ms, Rat	PY20	WB	HRP	50 µg/150 µg	**610011/12**
Phosphotyrosine	C, D, F, Hu, Ms, Rat	PY69	FCM, IF, IHC, IP, WB	Purified	1 mg	**610430**
Phosphotyrosine	C, D, F, Hu, Ms, Rat	RC20	WB	AKP	50 µg/150 µg	**610019/20**
Phosphotyrosine	C, D, F, Hu, Ms, Rat	RC20	IP, WB	Biotin	50 µg/150 µg	**610021/22**
Phosphotyrosine	C, D, F, Hu, Ms, Rat	RC20	WB	HRP	50 µg/150 µg	**610023/24**
Phosphotyrosine	C, D, F, Hu, Ms, Rat	Polyclonal	FCM, IF, IHC, IP, WB	Purified	50 µg/150 µg	**610009/10**
Phosphotyrosine	C, D, F, Hu, Ms, Rat	PY69	IP	Agarose	500 µl	**610015**

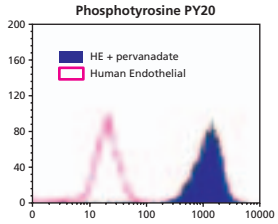


Figure 11. *Flow cytometric analysis using anti-Phosphotyrosine (610000) in human endothelial cells either untreated or treated with pervanadate.*

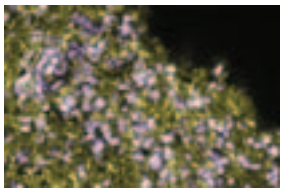


Figure 12. *Immunohistochemical staining using anti-Phosphotyrosine (610000) (green) in rabbit cerebrum.*

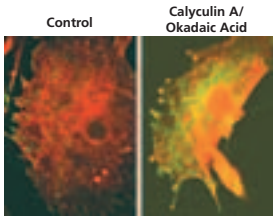


Figure 13. *Immunofluorescent staining using anti-Phosphotyrosine (610000) (green) and anti-Phosphoserine/threonine (red) in 3T3-L1 cells either untreated or treated with calyculin A and okadaic acid.*

Akt (PKBα)

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
Akt (PKBα)	D, Hu, Ms, Rat	7	IF, WB	Purified	50 µg/150 µg	**610836/37**
Akt (PKBα)	D, Hu, Ms, Rat	55	IF, WB	Purified	50 µg/150 µg	**610860/61**
Akt (PKBα)	D, Hu, Ms, Rat	2	IF, WB	Purified	50 µg/150 µg	**610876/77**
Akt (pS472/pS473), Phospho-Specific	Hu, Ms, Rat	104A282	IP, WB	Purified	50 µg	**550747**
Akt (PKBα) Sampler Kit			WB	Kit	10 µg each	**611437**

Applications: FCM: Flow Cytometry; IF: Immunofluorescence; IHC: Immunohistochemistry; IP: Immunoprecipitation; IVK: In Vitro Kinase Assay; WB: Western Blot
Reactivities: B: Bovine; Bab: Baboon; C: Chicken; D: Dog; E: Equine; F: Frog; G Pig: Guinea Pig; Hm: Hamster; Hu: Human; Mn: Monkey; Ms: Mouse; O: Ovine; P: Porcine; Rab: Rabbit

Antibodies for Phospho-Protein Analysis *(continued)*

Btk/Itk

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
Btk	Hu	53	IF, IHC, WB	Purified	50 µg/150 µg	**611116/17**
Btk	Hu	G149-11	WB	Purified	0.1 mg	**554239**
Btk (pY551)/Itk (pY511)		24a	IHC, WB	Purified	0.1 mg	**558034**

Caveolin

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
Caveolin	C, D, Hu, Ms, Rat	Polyclonal	FCM, IF, IHC, IP, WB	Purified	50 µg/150 µg	**610059/60**
Caveolin (pY14), Phospho-Specific	Hu, Ms, Rat	56	FCM, IF, IHC, WB	Purified	50 µg/150 µg	**611338/39**
Caveolin (pY14) Peptide				Peptide	100 µg	**611582**
Caveolin 1	C, Hu	2234	FCM, IF, IP, WB	Purified	50 µg/150 µg	**610493/94**
Caveolin 1	C, D, Hu, Ms, Rat	2297	FCM, IF, IHC, IP, WB	Purified	50 µg/150 µg	**610406/07**
Caveolin 1	D, Hu, Ms, Rab, Rat	C060	FCM, IF, IHC, IP, WB	Purified	50 µg/150 µg	**610057/58**
Caveolin 1	C	C20B	IF, WB	Purified	50 µg/150 µg	**610387/88**
Caveolin 2	Hu, Ms, Rat	65	WB	Purified	50 µg/150 µg	**610684/85**
Caveolin 2 (pY19), Phospho-Specific	Hu, Ms, Rat	PAb	WB	Purified	0.1 mg	**557859**
Caveolin Sampler Kit			WB	Kit	10 µg each	**611766**

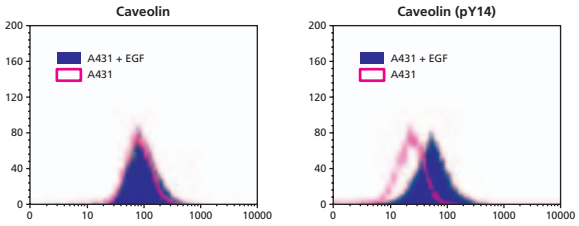


Figure 14. *Flow cytometric analysis using anti-Caveolin (610406) and anti-Caveolin (pY14) (611338) in A431 cells either untreated or treated with EGF.*

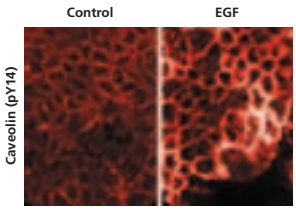


Figure 15. *Immunofluorescent staining using anti-Caveolin (pY14) (611338) in A431 cells either untreated or treated with EGF.*

Figure 16. *Western blot analysis using anti-Akt (pS472/ pS473/pS474) (559029) and anti-Caveolin (pY14) (611338) in A431 cells treated with EGF.*

c-Cbl

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
c-Cbl	C, D, Hu, Ms, Rat	17	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610441/42**
c-Cbl (pY700), Phospho-Specific	Hu	47	IF, IHC, WB	Purified	50 µg/150 µg	**612304/05**
B Cell Signaling Sampler Kit			IF, WB	Kit	10 µg each	**611661**

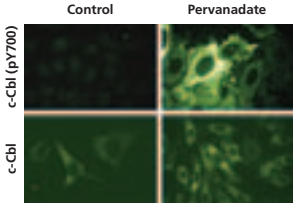


Figure 17. *Immunofluorescent staining using anti-c-Cbl (610441) and anti-c-Cbl (pY700) (612304) in human endothelial cells either untreated or treated with pervanadate.*

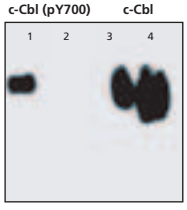


Figure 18. *Western blot analysis using anti-c-Cbl (610441) and anti-c-Cbl (pY700) (612304) in Jurkat T cells treated with either pervanadate (lanes 1 and 3) or alkaline phosphatase (lanes 2 and 4).*

Antibodies for Phospho-Protein Analysis *(continued)*

CD22

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
CD22	Hu	48	IHC, WB	Purified	50 µg/150 µg	**612462/63**
CD22 (pY828)	Hu	46	IHC, WB	Purified	0.1 mg	**558029**
CD22 (pY843)	Hu	12a	WB	Purified	0.1 mg	**558030**

Cdk1/Cdc2

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
Cdk1/Cdc2	Hu, Ms, Rat	1	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610037/38**
Cdk1/Cdc2	Hu, Ms	A-17	IP, WB	Purified	100 µg	**554161**
Cdk1/Cdc2		Polyclonal	IP, WB	Serum	.01 ml	**558900**
Cdk1/Cdk2	Hu	AN21.2	WB	Purified	50 µg/150 µg	**551526/25**
Cdk1/Cdc2 (pY15), Phospho-Specific	Hu	44	IHC, WB	Purified	50 µg/150 µg	**612306/07**
Cell Cycle I Sampler Kit			IF, WB	Kit	10 µg each	**611423**
Cell Cycle II Sampler Kit			IF, WB	Kit	10 µg each	**612744**

β-Dystroglycan

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
β-Dystroglycan	Hu	56	IHC, WB	Purified	50 µg/150 µg	**612090/91**
β-Dystroglycan (pY892), Phospho-Specific	Hu, Ms	27.1	FCM, IF, IHC, WB	Purified	50 µg/150 µg	**612524/25**

EGF Receptor

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
EGF Receptor	Hu, Ms	13	IF, IP, IHC, WB	Purified	50 µg/150 µg	**610016/17**
EGF Receptor	Hu, Ms	13	IF	FITC	50 µg/150 µg	**612554/55**
EGF Receptor	Hu	EGFR1	FCM, IF	Purified	100 µg	**555996**
EGF Receptor	Hu	EGFR1	FCM	PE	100 tests	**555997**
EGF Receptor (Activated Form)	Hu	74	IF, IP, IHC, WB	Purified	50 µg/150 µg	**610025/26**
EGFR Activation Sampler Kit			WB	Kit	10 µg each	**612476**

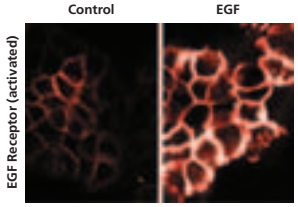


Figure 19. *Immunofluorescent staining using anti-Activated EGFR (610025) in A431 cells either untreated or treated with EGF.*

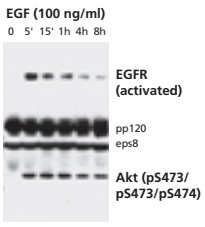


Figure 20. *Western blot analysis using anti-Activated EGFR (610025) and anti-Akt (pS472/pS473/ pS474) (559029) in A431 cells treated with EGF.*

Applications: FCM: Flow Cytometry; IF: Immunofluorescence; IHC: Immunohistochemistry; IP: Immunoprecipitation; IVK: In Vitro Kinase Assay; WB: Western Blot
Reactivities: B: Bovine; Bab: Baboon; C: Chicken; D: Dog; E: Equine; F: Frog; G Pig: Guinea Pig; Hm: Hamster; Hu: Human; Mn: Monkey; Ms: Mouse; O: Ovine; P: Porcine; Rab: Rabbit

Antibodies for Phospho-Protein Analysis *(continued)*

ERK1/2

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
ERK (pan ERK)	C, D, F, Hu, Ms, Rat	16	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610123/24**
ERK1	Hu, Ms	G262-118	WB	Purified	100 µg	**554100**
ERK1	B, C, D, F, Hu, Ms, Rat	MK1	FCM, IF, IHC, IP, WB	Purified	50 µg/150 µg	**610408/09**
ERK1	B, C, D, F, Hu, Ms, Rat	MK12	FCM, IF, IHC, IP, WB	Purified	50 µg/150 µg	**610030/31**
ERK1	B, C, D, F, Hu, Ms, Rat	MK12	IHC, WB	HRP	50 µg/150 µg	**610032/33**
ERK1/2 (pT202/pY204), Phospho-Specific	B, C, D, F, Hu, Ms, Rat	20A	FCM, IF, IHC, WB	Purified	50 µg/150 µg	**612358/59**
ERK2	C, D, F, Hu, Ms, Rat	33	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610103/04**
MAP Kinase Sampler Kit			WB	Kit	10 µg each	**611419**
MAP Kinase Activation Sampler Kit			WB	Kit	10 µg each	**612544**

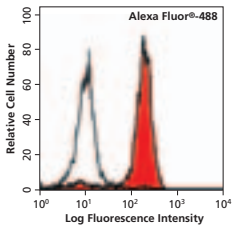


Figure 21. *Flow cytometric analysis using anti-ERK1/2 (pT202/pY204) Alexa Fluor® 488 (612592, clone 20A) in human peripheral blood mononuclear cells either untreated (unshaded) or treated (shaded) with PMA.*

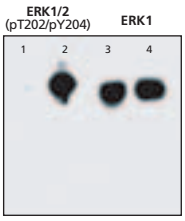


Figure 22. *Western blot analysis using anti-ERK1 (610030) and anti-ERK1/2 (pT202/pY204) (612358) in A431 cells either untreated (lanes 1 and 3) or treated with EGF (lanes 2 and 4).*

FAK

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
FAK	C, D, Hu, Ms, Rat	77	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610087/88**
FAK	F, Hu, Ms	Polyclonal	IP, WB	Serum	100 µg	**556368**
FAK (pY397), Phospho-Specific	Hu, Ms, Rat	14	IF, IHC, WB	Purified	50 µg/150 µg	**611722/23**
FAK (pY397), Phospho-Specific	Hu, Ms, Rat	18	IF, WB	Purified	50 µg/150 µg	**611806/07**
Focal Adhesion Sampler Kit			IF, WB	Kit	10 µg each	**611433**

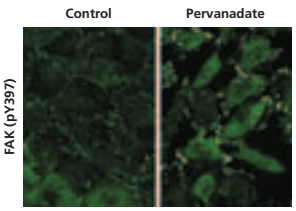


Figure 23. *Immunofluorescent staining using anti-FAK (pY397) (611722) in human endothelial cells either untreated or treated with pervanadate.*

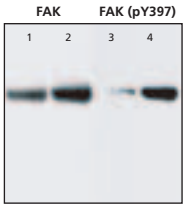


Figure 24. *Western blot analysis using anti-FAK (pY397) (611722) and anti-FAK (610087) in human fibroblast cells either untreated (lanes 1 and 3) or treated with pervanadate (lanes 2 and 4).*

Fyn

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
Fyn	Dog, Hu, Rat	25	IF, IHC, WB	Purified	50 µg/150 µg	**610163/610164**
Fyn (pY528)/c-Src (pY530)	Hu	31	IHC, WB	Purified	50 µg/150 µg	**612668/612669**

Antibodies for Phospho-Protein Analysis *(continued)*

GSK-3β

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
GSK-3β	C, D, Hu, Ms, Rat	7	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610201/02**
GSK-3β (pY216), Phospho-Specific	B, D, Hu, Ms, Rat	13a	IHC, WB	Purified	50 µg/150 µg	**612312/13**
PKB/Akt Sampler Kit			IF, WB	Kit	10 µg each	**611437**

IκBα

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
IκBα	Hu	25	IF, IP, WB	Purified	50 µg/150 µg	**610690/91**
IκBα	Hu	6A920	IP, WB	Purified	50 µg	**551819**
IκBα	Hu, Ms	Polyclonal	WB	Serum	.01 ml	**554135**
IκBα (pS32/pS36), Phospho-Specific	Hu	39A1431	WB	Purified	50 µg	**551818**
NF-κB Sampler Kit			IF, WB	Kit	10 µg each	**611665**

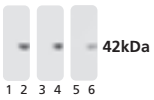


Figure 25. *Western blot analysis using anti-IκBα (pS32/pS36) (551818) in Jurkat T cells either untreated (lanes 1, 3, and 5) or treated with TNF (lanes 2, 4, and 6).*

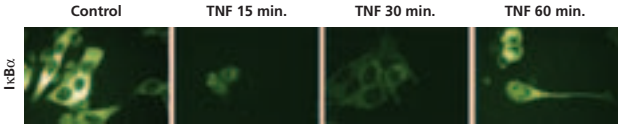


Figure 26. *Immunofluorescent staining using anti-IκBα (610690) shows transient degradation of IκBα in HeLa cells during treatment with TNF.*

Integrin β3

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
Integrin β3	Hu	1	IF, IHC, WB	Purified	50 µg/150 µg	**611140/41**
Integrin β3 (pY759), Phospho-Specific	Hu, Ms, Rat	7a	IHC, WB	Purified	50 µg/150 µg	**612528/29**
Integrin Sampler Kit			IF, WB	Kit	10 µg each	**611435**

JNK/SAPK

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
pan-JNK/SAPK1	C, D, F, Hu, Ms, Rat	37	IF, IHC, WB	Purified	50 µg/150 µg	**610627/28**
JNK (pT183/pY185), Phospho-Specific	Hu, Ms, Rat	41	FCM, IHC, WB	Purified	50 µg/150 µg	**612540/41**
JNK1	Hu	G151-333	IP, IVK, WB	Purified	50 µg/150 µg	**551196/97**
JNK1/JNK2	Hu	G151-666	IHC, IP, WB	Purified	100 µg	**554285**
Stress Response Sampler Kit			WB	Kit	10 µg each	**611442**

Jun

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
c-Jun (pS63)	Hu	2	WB	Purified	0.1 mg	**558036**
Jun	Hu, Ms, Rat	G56-206	WB	Purified	0.1 mg	**554083**
Jun	Bov, Chick, Dog, Hu, Ms, Rat	3	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610326/27**

Applications: FCM: Flow Cytometry; IF: Immunofluorescence; IHC: Immunohistochemistry; IP: Immunoprecipitation; IVK: In Vitro Kinase Assay; WB: Western Blot
Reactivities: B: Bovine; Bab: Baboon; C: Chicken; D: Dog; E: Equine; F: Frog; G Pig: Guinea Pig; Hm: Hamster; Hu: Human; Mn: Monkey; Ms: Mouse; O: Ovine; P: Porcine; Rab: Rabbit

Antibodies for Phospho-Protein Analysis *(continued)*

Lck

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
Lck	Hu, Ms, Rat	28	IF, IHC, WB	Purified	50 µg/150 ug	**610097/98**
Lck (pY505), Phospho-Specific	Hu, Ms, Rat	4	FCM, IHC, WB	Purified	50 µg/150 ug	**612390/91**
T Cell Signaling I Sampler Kit			IF, WB	Kit	10 µg each	**611662**

Neurofilament Proteins

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
NF-H	Rat	RFN402	WB	Purified	50 µg	**551349**
NF-H, Phospho-Specific	Rat	RNF404	WB	Purified	50 µg	**551348**
NF-H, Phospho-Specific	Rat	RNF405	WB	Purified	50 µg	**551958**
NF-M, Phospho-Specific	Rat	RNF403	WB	Purified	50 µg	**551957**
NF-M, Phospho-Specific	Rat	RNF406	WB	Purified	50 µg	**551962**

eNOS

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
eNOS/NOS Type III	Hu, Ms, Rat	3	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610296/97**
eNOS/NOS Type III	Hu	33	IF, IP, WB	Purified	50 µg/150 µg	**610427/28**
eNOS/NOS Type III	Hu, Ms, Rat	Polyclonal	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610298/99**
eNOS (pS1177), Phospho-Specific	B, D, Hu, Ms, Rat	19	FCM, IHC, WB	Purified	50 µg/150 µg	**612392/93**
eNOS (pT495), Phospho-Specific	B, D, Hu, Ms, Rat	31	IHC, WB	Purified	50 µg/150 µg	**612706/07**
eNOS (pS633), Phospho-Specific	Hu	37	WB	Purified	50 µg/150 µg	**612664/65**
NOS Sampler Kit			WB	Kit	10 µg each	**611426**

p38 MAPK

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
p38α (SAPK2a)	D, Hu, Ms, Rat	27	FCM, IF, IHC, WB	Purified	50 µg/150 µg	**612168/69**
p38 MAPK (pT180/pY182), Phospho-Specific	Hu, Ms, Rat	30	FCM, IF, IHC, WB	Purified	50 µg/150 µg	**612280/81**
p38 MAPK (pT180/pY182), Phospho-Specific	Hu, Ms, Rat	36	FCM, IF, IHC, WB	Purified	50 µg/150 µg	**612288/89**

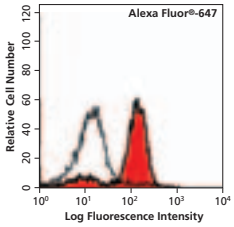


Figure 27. *Flow cytometric analysis using anti-p38 MAPK (pT180/pY182) Alexa Fluor® 647 (612595, clone 36) in human peripheral blood mononuclear cells either untreated (unshaded) or treated (shaded) with PMA.*

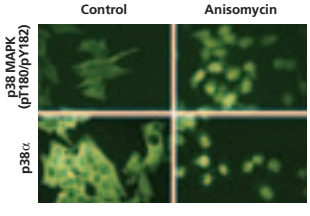


Figure 28. *Immunofluorescent staining using anti-p38α (611532) and anti-p38 MAPK (pT180/pY182) (612288) in HeLa cells either untreated or treated with anisomycin.*

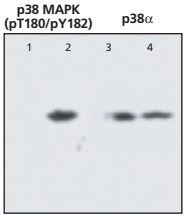


Figure 29. *Western blot analysis using anti-p38α (612168) and anti-p38 MAPK (pT180/pY182) (612288) in HeLa cells either untreated (lanes 1 and 3) or treated with anisomycin (lanes 2 and 4).*

Antibodies for Phospho-Protein Analysis *(continued)*

p90 RSK

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
Rsk	Chick, Dog, Hu, Ms, Rat	78	IF, IHC, WB	Purified	150 µg	**610226**
p90 RSK1 (pS380)	Hu	20a	WB	Purified	50 µg/150 µg	**612692/93**

p120 Catenin (pp120)

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
p120 Catenin (pp120)	C, D, Hu, Ms, Rat	98	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610133/34**
p120 Catenin (pp120)	C, D, Hu, Ms, Rat	98	IF	FITC	50 µg/150 µg	**610135/36**
p120 Catenin (pp120)	C, D, Hu, Ms, Rat	98	IF	TRITC	50 µg/150 µg	**610137/38**
p120 Catenin (pY96), Phospho-Specific	Hu, Ms, Rat	25a	IHC, WB	Purified	50 µg/150 µg	**612534/35**
p120 Catenin (pY228), Phospho-Specific	Hu, Ms, Rat	21a	FCM, IF, WB	Purified	50 µg/150 µg	**612536/37**
p120 Catenin (pY280), Phospho-Specific	Hu, Ms, Rat	18	IF, IHC, WB	Purified	50 µg/150 µg	**612538/39**
p120 Catenin (CpY291), Phospho-Specific	Hu, Ms, Rat	15a	IF, WB	Purified	50 µg/150 µg	**612690/91**

Paxillin

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
Paxillin	B, C, D, Hu, Ms, Rat	165	IF, IP, WB	Purified	50 µg/150 µg	**610619/20**
Paxillin	B, C, D, Hu, Ms, Rat	177	IF, WB	Purified	50 µg/150 µg	**610568/69**
Paxillin	C, D, Hu, Ms, Rat	349	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610051/52**
Paxillin	C, D, Hu, Ms, Rat	349	IF	FITC	50 µg/150 µg	**610053/54**
Paxillin	C, D, Hu, Ms, Rat	349	IF	TRITC	50 µg/150 µg	**610055/56**
Paxillin (pY118), Phospho-Specific	Hu, Ms	30	IHC, WB	Purified	50 µg/150 µg	**611724/25**
Focal Adhesion Sampler Kit			IF, WB	Kit	10 µg each	**611433**

Phospholipase Cγ1

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
Phospholipase Cγ1	C, D, Hu, Ms, Rat	10	FCM, IF, IHC, IP, WB	Purified	50 µg/150 µg	**610027/28**
Phospholipase Cγ1 (pY783), Phospho-Specific	B, Hu, Ms, Rat	27	IHC, WB	Purified	50 µg/150 µg	**612464/65**

$PKA_{RII\beta}$

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
$PKA_{RII\beta}$	C, D, Hu, Ms, Rat	45	IF, IHC, WB	Purified	50 µg/150 µg	**610625/26**
$PKA_{RII\beta}$ (pS114), Phospho-Specific	Hu, Ms, Rat	24	FCM, WB	Purified	50 µg/150 µg	**612572/73**
$PKA_{RII\beta}$ (pS114), Phospho-Specific	Hu, Ms, Rat	47	FCM, IHC, WB	Purified	50 µg/150 µg	**612550/51**
PKA Sampler Kit			IF, WB	Kit	10 µg each	**611420**

Applications: FCM: Flow Cytometry; IF: Immunofluorescence; IHC: Immunohistochemistry; IP: Immunoprecipitation; IVK: In Vitro Kinase Assay; WB: Western Blot
Reactivities: B: Bovine; Bab: Baboon; C: Chicken; D: Dog; E: Equine; F: Frog; G Pig: Guinea Pig; Hm: Hamster; Hu: Human; Mn: Monkey; Ms: Mouse; O: Ovine; P: Porcine; Rab: Rabbit

Antibodies for Phospho-Protein Analysis *(continued)*

PKC

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
PKC	Hu, Ms, Rat, Bov, Chick, Rab	MC5	IF, IHC(Fr), IP, WB	Purified	0.1 mg	**554207**
PKCα	Chick, Dog, Frog, Hu, Ms, Rat	3	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610107/08**
PKCα (pT638)	Hu	35	IHC, WB	Purified	50 µg/150 µg	**612698/99**
PKCβ	Chick, Hu, Ms, Rat	36	IP, WB	Purified	50 µg/150 µg	**610127/28**
PKCδ	Hu, Ms, Rat	14	IF, IHC, WB	Purified	50 µg/150 µg	**610397/98**
PKCε	Chick, Dog, Hu, Ms, Rat	21	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610085/86**
PKCγ	Ms, Rat	20	WB	Purified	50 µg/150 µg	**611158/59**
PKCη	Hu, Ms	31	IF, WB	Purified	50 µg/150 µg	**610814/15**
PKCι	Chick, Dog, Hu, Ms, Rat	23	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610175/76**
PKCι	Dros	23	IF, IP, WB	Purified	50 µg	**612645**
PKCλ	Chick, Dog, Hu, Ms, Rat	41	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610207/08**
PKCθ	Hu	27	IF, IP, IHC, WB	Purified	50 µg/150 µg	**610089/90**
PKCθ	Hu	27	WB	HRP	50 µg/150 µg	**610091/92**
PKCθ (pT538)	Hu	19	IHC, WB	Purified	50 µg/150 µg	**612734/35**
PKC Sampler Kit			IF, WB	Purified	10 µg each	**611421**

Ras-GAP

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
Ras-GAP	Chick, Dog, Hu, Ms, Rat	Polyclonal	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610043/44**
Ras-GAP	Chick, Dog, Frog, Hu, Ms, Rat	13	IF, IHC, WB	Purified	50 µg/150 µg	**610040/41**
Ras-GAP (pY460)	Hu	19A	IHC, WB	Purified	50 µg/150 µg	**612736/37**

Rb

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
Rb (a.a. 1-240)	Hu	G99-2005	IP, WB	Purified	100 µg	**554162**
Rb (a.a. 300-380)	C,Hu	C36	GS, IHC, IP, WB	Purified	100 µg	**554142**
Rb (a.a. 300-380)	Hu	G3-349	IP, WB	Purified	100 µg	**554140**
Rb (a.a. 300-380)	Hu	G4-340	IP, WB	Purified	100 µg	**554141**
Rb (a.a. 300-508)	C, Hu	2	IF, IHC, WB	Purified	50 µg/150 µg	**610884/85**
Rb (a.a. 332-344)	Hu, Ms, Rat	G3-245	FCM, GS, IF, IHC, IP, WB	Purified	100 µg	**554136**
Rb (specially formatted for IHC)	Hu, Ms, Rat	G3-245	IHC	Purified	1 ml	**550830**
Rb mAb / Isotype Reagent Set : FITC	Hu, Ms, Rat	G3-245	FCM	FITC Set	100 Tests	**556538**
Rb mAb / Isotype Reagent Set : PE	Hu, Ms, Rat	G3-245	FCM	PE Set	100 Tests	**556539**
Rb (a.a. 393-572)	C, Hu, Ms	XZ104	IP	Purified	100 µg	**554143**
Rb (a.a. 443-622)	C, F, Hu, Ms	XZ55	GS, IP, WB	Purified	100 µg	**554144**
Rb (a.a. 444-535)	C, Hu	XZ91	IF, IP, WB	Purified	100 µg	**554145**
Rb (a.a. 622-665)	C, Hu, Ms	XZ133	IP	Purified	100 µg	**554146**
Underphosphorylated Rb (a.a. 514-610)	Hu, Ms	G99-549	IHC, IP, WB	Purified	100 µg	**554164**
Underphosphorylated Rb mAb / Isotype Reagent Set	Hu	G99-549	FCM	FITC Set	100 Tests	**550501**
Underphosphorylated Rb mAb / Isotype Reagent Set	Hu	G99-549	FCM	PE Set	100 Tests	**550502**

RNA Polymerase II

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
RNA Polymerase II	Hu	CTD4H8	WB	Purified	150 µg	**552042**
RNA Polymerase II, Phospho-Specific	Hu	CTD8A7	WB	Purified	150 µg	**552041**

Antibodies for Phospho-Protein Analysis *(continued)*

Stats

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
Stat1 (C-terminus)	C, D, Hu, Ms, Rat	42	IF, IP, IHC, WB	Purified	50 µg/150 µg	**610185/86**
Stat1 (N-terminus)	C, D, F, Hu, Ms, Rat	1	IF, IHC, IP, WB	Purified	50 µg/150 µg	**610115/16**
Stat1 (N-terminus)	C, D, F, Hu, Ms, Rat	1	WB	HRP	50 µg/150 µg	**610117/18**
Stat1 (N-terminus)	C, D, F, Hu, Ms, Rat	Polyclonal	IF, IP, WB	Purified	50 µg/150 µg	**610119/20**
Stat1 (pY701), Phospho-Specific	Hu, Ms, Rat	4a	FCM, IF, IHC, IP, WB	Purified	50 µg/150 µg	**612232/33**
Stat1 (pY701), Phospho-Specific	Hu, Ms, Rat	14	FCM, IF, IP, WB	Purified	50 µg/150 µg	**612132/33**
Stat3	C, D, F, Hu, Ms, Rat	84	IHC, IP, WB	Purified	50 µg/150 µg	**610189/90**
Stat3 (pY705), Phospho-Specific	Hu, Ms, Rat	4	FCM, IHC, WB	Purified	50 µg/150 µg	**612356/57**
Stat3 (pS727), Phospho-Specific	Hu, Ms, Rat	49	IF, IHC, WB	Purified	50 µg/150 µg	**612542/43**
Stat4	Ms, Rat	8	IHC, WB	Purified	50 µg/150 µg	**610926/27**
Stat4 (pY693), Phospho-Specific	Hu	38	FCM, IF, IHC, WB	Purified	50 µg/150 µg	**612738/39**
Stat5	D, Hu, Ms, Rat	89	FCM, IF, IHC, WB	Purified	50 µg/150 µg	**610191/92**
Stat5 (pY694), Phospho-Specific	B, Hu, Ms, Rat	47	FCM, IHC, WB	Purified	50 µg/150 µg	**611964/65**
Stat5 (pY694), Phospho-Specific	B, Hu, Ms, Rat	Polyclonal	FCM, IF, WB	Purified	50 µg/150 µg	**611818/19**
Stat5A	D, Hu, Ms, Rat	51	IP, WB	Purified	50 µg/150 µg	**611834/35**
Stat5A	Hu	Polyclonal	IP, WB	Purified	50 µg	**556516**
Stat5B	Hu, Ms, Rat	Polyclonal	IP, WB	Purified	50 µg	**556517**
Stat6	Hu, Ms, Rat	23	IF, IHC, WB	Purified	50 µg/150 µg	**611290/91**
Stat6 (pY641), Phospho-Specific	Hu	18	FCM, IF, IHC, WB	Purified	50 µg/150 µg	**611566/67**
Stat6 (pY641), Phospho-Specific	Hu	Polyclonal	FCM, IF, WB	Purified	50 µg/150 µg	**611820/21**
Stat Sampler Kit			WB	Kit	10 µg each	**611422**
Stat Activation Sampler Kit			FCM, IF, WB	Kit	10 µg each	**612477**

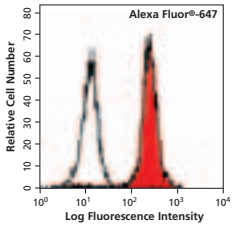


Figure 30. *Flow cytometric analysis using anti-Stat1 (pY701) Alexa Fluor® 647 (612597, clone 4A) in U937 cells either untreated (unshaded) or treated (shaded) with recombinant human IFN-g.*

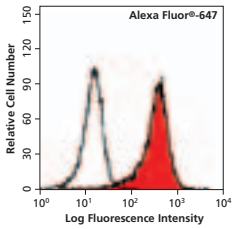


Figure 31. *Flow cytometric analysis using anti-Stat6 (pY641) Alexa Fluor® 647 (612601, clone 18) in human endothelial cells either untreated (unshaded) or treated (shaded) with recombinant human IL-4.*

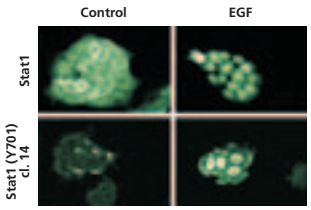


Figure 32. *Immunofluorescent staining using anti-Stat1 (610115) and anti-Stat1 (pY701) (612132) in A431 cells either untreated or treated with EGF.*

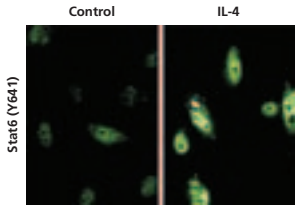


Figure 33. *Immunofluorescent staining using anti-Stat6 (pY641) (611566) in human endothelial cells either untreated or treated with IL-4.*

Figure 34. *Western blot analysis using anti-Stat1 (610115) and anti-Stat1 (pY701) (612132) in A431 cells either untreated (lanes 1 and 3) or treated with EGF (lanes 2 and 4).*

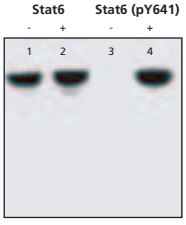


Figure 35. *Western blot analysis using anti-Stat6 (611290) and anti-Stat6 (pY641) (611566) in human endothelial cells either untreated (lanes 1 and 3) or treated with IL-4 (lanes 2 and 4).*

ZAP-70/Syk

DESCRIPTION	REACT	CLONE	APPS	FORMAT	SIZE	CAT. NO.
ZAP-70 Kinase	C, Hu, Ms, Rat	29	IF, IHC, IHC, WB	Purified	50 µg/150 µg	**610239/40**
ZAP-70 Kinase	C, Hu, Ms, Rat	29	IF	FITC	50 µg/150 µg	**612588/89**
ZAP-70 Kinase (pY319)/Syk(pY352), Phospho-Specific	Hu, Ms, Rat	17a	FCM, IHC, WB	Purified	50 µg/150 µg	**612574/75**

Applications: FCM: Flow Cytometry; IF: Immunofluorescence; IHC: Immunohistochemistry; IP: Immunoprecipitation; IVK: In Vitro Kinase Assay; WB: Western Blot
Reactivities: B: Bovine; Bab: Baboon; C: Chicken; D: Dog; E: Equine; F: Frog; G Pig: Guinea Pig; Hm: Hamster; Hu: Human; Mn: Monkey; Ms: Mouse; O: Ovine; P: Porcine; Rab: Rabbit

Activation-State Antibody Sampler Kits

BD™ Antibody Sampler Kits are a convenient, cost-effective means of evaluating several proteins without having to purchase individual antibodies from a variety of sources. The kits contain 10 µg of each antibody, all within a related field of research, plus the appropriate Western blot positive control lysates to verify your results. In addition, each antibody is available in larger sizes for your follow-up studies.

- *Cost-effective*
- *Kits contain 10 µg of each antibody plus a Western blot positive control lysate*
- *Phospho and non-phospho antibody pairs provided to study total protein expression*
- *Kits feature well-characterized, high-quality monoclonal antibodies for greater specificity*
- *Antibodies are available in larger sizes for follow-up studies*

Our newest releases feature antibodies to both phospho- and non-phospho proteins involved in three significant signaling systems:

- *EGFR Activation*
- *MAP Kinase Activation*
- *Stat Activation*

In addition, we offer Antibody Sampler Kits for the study of Akt/PKBα, B Cell Signaling, Caveolins, Cell Cycle, Focal Adhesion, Integrins, MAP Kinases, NF-κB, NOS, PKA, PKC, PTP, Ras, Stats, T Cell Signaling, plus the option of formulating your own Custom Antibody Sampler Kit. For more information, please call **877.232.8995** (US) or contact your local BD Biosciences office. We also invite you to visit us on-line at **www.bdbiosciences.com**

EGFR Activation Sampler Kit - Cat. No. 612476 – Price

COMPONENTS	CLONE	APPS	REACTIVITY	CAT. NO.
Akt/PKBα	7	IF, WB	Dog, Hu, Ms, Rat	**610836/37**
Akt/PKBα (pS472/pS473)	104A282	WB	Hu, Ms, Rat	**550747**
EGF Receptor	13	IF, IP, WB	Hu, Ms	**610016/17**
EGF Receptor (Activated Form)	74	IF, IP, WB	Hu	**610025/26**
ERK1	MK12	FCM, IF, IHC, IP, WB	B, C, Dog, Hu, Ms, Rat	**610030/31**
ERK1/2 (pT202/pY204)	20A	FCM, IF, WB	B, C, D, F, Hu, Ms, Rat	**612358/59**
Stat1 (N-terminus)	1	IF, IHC, IP, WB	C, Dog, Hu, Ms, Rat	**610115/16**
Stat1 (pY701)	4a	IF, IP, WB	Hu, Ms, Rat	**612232/33**

MAP Kinase Activation Sampler Kit - Cat. No. 612544 – Price

COMPONENTS	CLONE	APPS	REACTIVITY	CAT. NO.
ERK1	MK12	FCM, IF, IHC, IP, WB	B, C, Dog, Hu, Ms, Rat	**610030/31**
ERK1/2 (pT202/pY204)	20A	FCM, IF, WB	B, C, D, F, Hu, Ms, Rat	**612358/59**
pan-JNK/SAPK1	37	IF, IHC, WB	C, Dog, Hu, Ms, Rat	**610627/28**
JNK (pT183/pY185)	41	FCM, WB	Hu, Ms, Rat	**612540/41**
p38-α/SAPK2a	27	IF, WB	Dog, Hu, Ms, Rat	**612168/69**
p38 MAPK (pT180/pY182)	30	FCM, IF, WB	Hu, Ms, Rat	**612280/81**

Stat Activation Sampler Kit - Cat. No. 612477 – Price

COMPONENTS	CLONE	APPS	REACTIVITY	CAT. NO.
Stat1 (C-terminus)	42	IF, IP, WB	C, Dog, Hu, Ms, Rat	**610185/86**
Stat1 (pY701)	14	FCM, IF, IP, WB	Hu, Ms, Rat	**612132/33**
Stat3	84	IF, IP, WB	C, Dog, Hu, Ms, Rat	**610189/90**
Stat3 (pY705)	4	FCM, WB	Hu, Ms, Rat	**612356/57**
Stat5	89	IF, IHC, WB	Dog, Hu, Ms, Rat	**610191/92**
Stat5 (pY694)	47	FCM, WB	B, Hu, Ms, Rat	**611964/65**
Stat6	23	IF, WB	Hu, Ms, Rat	**611290/91**
Stat6 (pY641)	18	FCM, IF, WB	Hu	**611566/67**

FCM: Flow Cytometry; **IF:** Immunofluorescence Microscopy; **IHC:** Immunohistochemistry; **IP:** Immunoprecipitation; **WB:** Western Blot; **B:** Bovine; **C:** Chicken; **Hu:** Human; **Ms:** Mouse

Treated Lysates for Western Blot

DESCRIPTION	APPS	TISSUE TYPE	SIZE	CAT. NO.
A431 + EGF Cell Lysate	WB	Epidermoid Carcinoma	500 µg	**611448**
A431 + EGF AP Ctrl Lysate	WB	Epidermoid Carcinoma	500 µg	**612190**
HE + Pervanadate Cell Lysate	WB	Endothelium	500 µg	**611667**
HeLa + Anisomycin Lysate	WB	Epitheloid Cervical Carcinoma	500 µg	**611692**
HeLa Pervanadate Ctrl Lysate	WB	Epitheloid Cervical Carcinoma	500 µg	**612014**
HeLa + Pervanadate Lysate	WB	Epitheloid Cervical Carcinoma	500 µg	**612015**
HeLa + Pervanadate AP Ctrl Lysate	WB	Epitheloid Cervical Carcinoma	500 µg	**612016**
HeLa + Pervanadate + AP Lysate	WB	Epitheloid Cervical Carcinoma	500 µg	**612017**
HepG2 IL-6 Ctrl Lysate	WB	Hepatocellular Carcinoma	500 µg	**612066**
HepG2 + IL-6 (15') Lysate	WB	Hepatocellular Carcinoma	500 µg	**612067**
Hs68 + Pervanadate Lysate	WB	Fibroblast	500 µg	**612187**
Hs68 + Pervanadate AP Ctrl Lysate	WB	Fibroblast	500 µg	**612188**
Hs68 + Pervanadate + AP Lysate	WB	Fibroblast	500 µg	**612189**
Apoptotic Jurkat Lysate Set 1	WB	T Cell Leukemia	500 µg each	**550959**
Jurkat + Pervanadate Lysate	WB	T Cell Leukemia	500 µg	**611755**
Jurkat + Pervanadate AP Ctrl Lysate	WB	T Cell Leukemia	500 µg	**612192**
Jurkat + Pervanadate + AP Lysate	WB	T Cell Leukemia	500 µg	**612193**
Mouse Macrophage + IFNγ/LPS	WB	Macrophage	500 µg	**611473**

Regional Offices

Asia Pacific
BD Singapore
Tel 65.6861.0633
Fax 65.6860.1593

Australia/New Zealand
Australia
Tel 61.2.8875.7000
Toll Free: 1800 656 100
Fax 61.2.8875.7200
bd_anz@bd.com
New Zealand
Toll Free: 0800 572.468
Tel 64.9.574.2468
Fax 64.9.574.2469
bd_anz@bd.com

Canada
BD Biosciences
Toll free 888.259.0187
Tel 905.542.8028
Fax 888.229.9918
canada@bd.com

Europe
Belgium
Tel 32.53.720.550
Fax 32.53.720.549
customer_service_bdbelgium@europe.bd.com

Japan
Nippon Becton Dickinson
Toll free 0120.8555.90
Tel 81.24.593.5405
Fax 81.24.593.5761

United States
BD Biosciences
Customer/Technical Service
Toll free 877.232.8995
Bioimaging Systems
Fax 301.340.9775
Discovery Labware
Fax 978.901.7493
Immunocytometry Systems
Fax 800.325.9637
Pharmingen
Fax 800.325.9637
www.bdbiosciences.com

Local Offices and Distributors

Argentina/Paraguay/Uruguay
Tel 54.11.4551.7100
Fax 54.11.4551.7400
crc_argentina@bd.com

Austria
Tel 43.1.706.36.60.20
Fax 43.1.706.36.60.11
customerservice.bdb.at@europe.bd.com

Brazil
Unidade Biosciences
Tel 55.11.5185.9995
Fax 55.11.5185.9895
biosciences@bd.com.br

Central America/Caribbean
Tel 502.2 440 4195
Fax 502.2 440 3570

Chile
Tel 56.2 460.0380 x16
Fax 56.2 460.0306

China
Tel 8610.6418.1608
Fax 8610.6418.1610

Colombia
Tel 57.1.572.4060 x244
Fax 57.1.572.3650

Denmark
Tel 45.43.43.45.66
Fax 45.43.43.41.66
bdbnordic@europe.bd.com

East Africa
Tel 254.2.341157
Fax 254.2.341161
bd@africaonline.co.ke

Eastern Europe
Tel 49.6221.305.161
Fax 49.6221.305.418
bdb.ema@europe.bd.com

Egypt
Tel 202.268.0181
Fax 202.266.7562

Finland
Tel 358.9.88.70.7832
Fax 358.9.88.70.7817
bdbnordic@europe.bd.com

France
Tel 33.4.76.68.36.36
Fax 33.4.76.68.35.06
customerservice.bdb.france@europe.bd.com

Germany
Tel 49.6221.305.551
Fax 49.6221.303.609
customerservice.bdb.de@europe.bd.com

Greece
Tel 30.1.940.77.41
Fax 30.1.940.77.40

Hong Kong
Tel 852.2575.8668
Fax 852.2803.5320

Hungary
Tel 36.1.345.7090
Fax 36.1.345.7093
bdb.ema@europe.bd.com

India
Tel 91.124.238.3566.70
Fax 91.124.238.3225

Indonesia
Tel 62.21.577.1920
Fax 62.21.577.1925

Italy
Tel 39.02.48.240.1
Fax 39.02.48.20.33.36

Japan
Fujisawa Pharmaceutical Co., Ltd. (Reagents from Immunocytometry Systems & Pharmingen)
Tel 81.6.6206.7890
Fax 81.6.6206.7934

Korea
Tel 822.3404.3700
Fax 822.557.4048

Malaysia
Tel 603.7725.5517
Fax 603.7725.4772

Mexico
Tel 52.55.5999.8296
Fax 52.55.5999.8288

Middle East
Tel 971.4.337.95.25
Fax 971.4.337.95.51
bdb.ema@europe.bd.com

The Netherlands
Tel 31.20.582.94.20
Fax 31.20.582.94.21
customer_service_bdholland@europe.bd.com

North Africa
Tel 33.4.76.68.35.03
Fax 33.4.76.68.35.44
bdbiosciences_maghreb@europe.bd.com

Norway
Laborel S/A
Tel 47.23.05.19.30
Fax 47.23.05.19.31

Pakistan
Tel 92.42.5718051
Fax 92.42.5718056
bdb_pak@bd.com

Peru/Bolivia/Ecuador
Tel 51.1.430.0323
Fax 51.1.430.1077

Philippines
Tel 632.815.8981
Fax 632.815.6644

Poland
Tel 48.22.651.75.88
Fax 48.22.651.75.89
bdb.ema@europe.bd.com

Portugal
Enzifarma
Tel 351.21.421.93.30
Fax 351.21.421.93.39

South Africa
Tel 27.11.807.15 31
Fax 27.11.807.19 53
bdb.ema@europe.bd.com

Spain
Tel 34.902.27.17.27
Fax 34.91.848.81.04

Sweden
Tel 46.8.775.51.10
Fax 46.8.775.51.11
bdbnordic@europe.bd.com

Switzerland
Tel 41.61.485.22.22
Fax 41.61.485.22.00
customerservice.bdb.ch@europe.bd.com

Taiwan
Tel 8862.2722.5660
Fax 8862.2725.1768

Thailand
Tel 662.643.1374
Fax 662.643.1381

Turkey
Tel 90.212.328.2720
Fax 90.212.328.2730
bdb.ema@europe.bd.com

United Kingdom & Ireland
Tel 44.1865.78.16.88
Fax 44.1865.78.16.27
BDUK_CustomerService@europe.bd.com

Venezuela
Tel 58.212.241.3412 x248
Fax 58.212.241.7389

West Africa
Sobidis
Tel 225.20.33.40.32
Fax 225.20.33.40.28

BD

04-7900030-22B1