Technical Data Sheet

Purified Mouse Anti-Cytochrome c

Product Information

Material Number:	**556432**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	6H2.B4
Immunogen:	Rat cytochrome c
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	Confirmed by immunoprecipitation and Bioimaging: Human Confirmed during development by immunoprecipitation: Mouse, Rat
Target MW:	15 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

A cytochrome is an electron-transporting protein that contains a heme prosthetic group. Cytochromes have been known to be essential components of the mitochondrial respiratory chain since 1925. The iron atom of the heme group in cytochromes alternates between a reduced ferrous (+2) state and an oxidized ferric (+3) state during electron transport in oxidative phosphorylation. Cytochromes are classified into four groups (a, b, c and d) according to spectrochemical characteristics, and there are five cytochromes between coenzyme QH2 and O2 in the electron transport chain. Cytochrome c is a water-soluble protein that either promotes cell survival or death, depending upon its intracellular location. In healthy cells, it is a peripheral membrane protein of the mitochondria that transports electrons from the coenzyme QH2 cytochrome c reductase complex to the cytochrome c oxidase complex. When proapoptotic stimuli induce breakdown of the mitochondria, cytochrome c is released to the cytosol where it functions in the activation of caspases that trigger apoptosis.

The 6H2.B4 monoclonal antibody has been reported to recognize the native and not the denatured form of rat, mouse, and human cytochrome c. Furthermore, studies utilizing competitive ELISA indicate that mAb 6H2.B4 binds to a region around residue 62 of rat cytochrome c.

The 6H2.B4 monoclonal antibody is not useful for western blot analysis. For the western blot application, clone 7H8.2C12 (cat.no. 556433) would be recommended. Suggested positive controls for detecting cytochrome c include P388D mouse lymphoma cells (ATCC CCL 46), HeLa human carcinoma cells (ATCC CCL2), Jurkat T leukemia cells (ATCC TIB152) and NIH 3T3 mouse fibroblast cells (ATCC CRL 1658). This antibody is routinely tested by immunoprecipitation and immunofluorescent imaging. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4° C.

Application Notes

Application

Bioimaging	Routinely Tested
Immunoprecipitation	Routinely Tested
Western blot	Not Recommended

Recommended Assay Procedure:

Methanol Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

Triton-X 100 Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well

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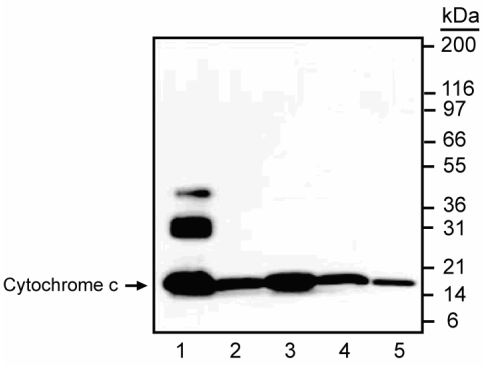
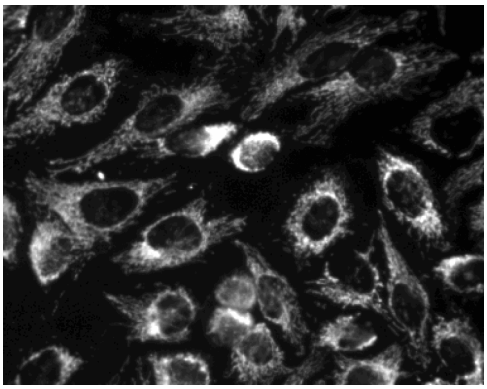
blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.



Immunoprecipitation analysis of Cytochrome c. *Clone 6H2. B4 (Cat. No. 556432) was used at 2 µg/ml to immunoprecipitate Cytochrome c from P388D1 mouse lymphoma (lane 2), HeLa human carcinoma (lane 3), Jurkat T leukemia (lane 4), and NIH/3T3 mouse fibroblast (lane 5) cell lysates. Cytochrome c was detected by Western blot analysis using clone 7H8.2C12 (Cat. No. 556433) (lanes 1-5). Purified rat Cytochrome c was used as a protein standard in lane 1. Cytochrome c is detected at a molecular weight of ~15 kDa. The upper bands in lane 1 represent dimers or multimers of purified Cytochrome C.*



Immunofluorescent staining of HeLa cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~10,000 cells per well. After overnight incubation, cells were stained using the Triton X100 fix/perm protocol (above) and the anti-Cytochrome C antibody. The second-step reagent was Alexa Fluor 488 anti-mouse IgG (Invitrogen). The image was taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained U2OS and A549 cells and worked with both the Triton X100 and Methanol fix/perm protocols (see Recommended Assay Procedure).*

Suggested Companion Products

Catalog Number	Name	Size	Clone
353219	BD Falcon™ 96-well Imaging Plate	1 box	(none)
611452	NIH 3T3 Cell Lysate	500 µg	(none)
611449	HeLa Cell Lysate	500 µg	(none)
611451	Jurkat Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Igs	1.0 ml	(none)
556433	Purified Mouse Anti-Cytochrome C	0.1 mg	7H8.2C12

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

References

Arnoult D, Grodet A, Lee Y-J, Estaquier J, Blackstone C. Release of OPA1 during apoptosis participates in the rapid and complete release of cytochrome c and subsequent mitochondrial fragmentation. *J Biol Chem.* 2005; 280(42):35742-35750.(Clone-specific: Immunofluorescence)

Garrido C, Galluzzi L, Brunet M, Puig PE, Didelot C, Kroemer G. Mechanisms of cytochrome c release from mitochondria. *Cell Death Differ.* 2006; 13:1423-1433. (Biology)

Goshorn SC, Retzel E, Jemmerson R. Common structural features among monoclonal antibodies binding the same antigenic region of cytochrome c. *J Biol Chem.* 1991; 266(4):2134-2142.(Immunogen: ELISA)

Jemmerson R, Johnson JG. different functional boundaries for the major antigenic region of two cytochromes c. *Proc Natl Acad Sci U S A.* 1991; 88:4428-4432. (Immunogen: ELISA)

Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell.* 1996; 86(1):147-157.(Clone-specific: Immunoprecipitation)

Rossé T, Olivier R, Monney L, et al. Bcl-2 prolongs cell survival after Bax-induced release of cytochrome c. *Nature.* 1998; 391(6666):496-499.(Clone-specific: Immunofluorescence)