Technical Data Sheet

Alexa Fluor® 488 Mouse anti-Cytochrome c

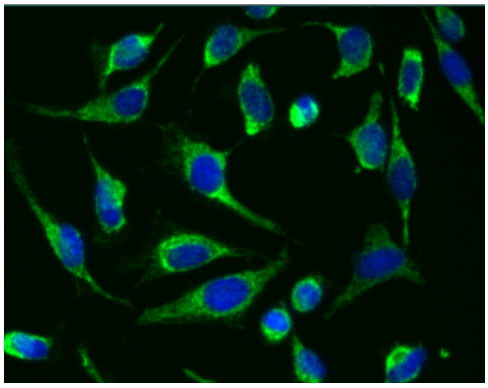
Product Information

Material Number:	**560263**
Size:	100 tests
Vol. per Test:	5 µl
Clone:	6H2.B4
Immunogen:	Rat Cytochrome c
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	Confirmed by Bioimaging: Human Confirmed by immunoprecipitation using the purified antibody (Cat. no. 556432): Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤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.



Immunofluorescent staining of human cell lines. *HeLa cells (ATCC CCL-2) were seeded in a 96-well imaging plate (Cat. No. 353219) at ~10,000 cells per well. After overnight incubation, the cells were fixed, permeabilized with Triton™ X-100, stained with Alexa Fluor® 488 Mouse anti-Cytochrome c (pseudo colored green), and the nuclei were counterstained with Hoechst 33342 (pseudo colored blue) according to the Recommended Assay Procedure. The images were captured on a BD Pathway™ 435 Cell Analyzer with a 20x objective and merged using BD Attovision™ software. This antibody also stains A549 (ATCC CCL-185) and U-2 OS (ATCC HTB-96) cells using Triton™ X-100, cold methanol, or Saponin for permeabilization (see Recommended Assay Procedure).*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Bioimaging	Routinely Tested

Recommended Assay Procedure:

1. Seed the cells in appropriate culture medium at an appropriate cell density in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219), and culture overnight to 48 hours.
2. Remove the culture medium from the wells, and wash (one to two times) with 100 µl of 1× PBS.

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3. Fix the cells by adding 100 µl of fresh 3.7% Formaldehyde in PBS or BD Cytofix™ fixation buffer (Cat. No. 554655) to each well and incubating for 10 minutes at room temperature (RT).
4. Remove the fixative from the wells, and wash the wells (one to two times) with 100 µl of 1× PBS.
5. Permeabilize the cells using either cold methanol (a), Triton™ X-100 (b), or Saponin (c):
    a. Add 100 µl of -20°C 90% methanol or -20°C BD™ Phosflow Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.
    b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
    c. Add 100 µl of 1× Perm/Wash buffer (Cat. No. 554723) to each well and incubate for 15 to 30 minutes at RT. Continue to use 1× Perm/Wash buffer for all subsequent wash and dilutions steps.
6. Remove the permeabilization buffer from the wells, and wash one to two times with 100 µl of appropriate buffer (either 1× PBS or 1× Perm/Wash buffer, see step 5.c.).
7. Optional blocking step: Remove the wash buffers, and block the cells by adding 100 µl of blocking buffer BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) or 3% FBS in appropriate dilution buffer to each well and incubating for 15 to 30 minutes at RT.
8. Dilute the antibody to its optimal working concentration in appropriate dilution buffer. Titrate purified (unconjugated) antibodies and second-step reagents to determine the optimal concentration. If using a Bioimaging Certified antibody conjugate, dilute it 1:10.
9. Add 50 µl of diluted antibody per well and incubate for 60 minutes at RT. Incubate in the dark if using fluorescently labeled antibodies.
10. Remove the antibody, and wash the wells three times with 100 µl of wash buffer. An optional detergent wash (100 µl of 0.05% Tween in 1× PBS) can be included prior to the regular wash steps.
11. If the antibody being used is fluorescently labeled, then move to step 12. Otherwise, if using a purified unlabeled antibody, repeat steps 8 to 10 with a fluorescently labeled second-step reagent to detect the purified antibody.
12. After the final wash, counter-stain the nuclei by adding 100 µl of a 2 µg/ml solution of Hoechst 33342 (eg, Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
13. View and analyze the cells on an appropriate imaging instrument. Recommended filters for the BD Pathway™ instruments are:

Instrument	*Excitation*	*Emission*	*Dichroic*
BD Pathway 855	488/10	515 LP	Fura/FITC
BD Pathway 435	482/35	536/40	FF506

Suggested Companion Products

Catalog Number	Name	Size	Clone
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. This reagent has been pre-diluted for use at the recommended Volume per Test when following the Recommended Assay Procedure. A Test is typically ~10,000 cells cultured in a well of a 96-well imaging plate.
3. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
4. 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.
5. Triton is a trademark of the Dow Chemical Company.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References

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