Technical Data Sheet

Purified Mouse Anti-Human Caspase-2 w/Control

Product Information

Material Number:	**551093**
Reactivity:	QC Testing: Human
Component:	**51-1395GR**
Description:	Purified Mouse Anti-Human Caspase-2 (Ich-1)
Size:	50 µg (1 ea)
Concentration:	0.25 mg/ml
Clone Name:	G310-1248
Immunogen:	Human Caspase-2 (short)
Isotype:	Mouse IgG1
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.
Component:	**51-16526N**
Description:	Jurkat Cell Lysate
Size:	50 µg (1 ea)
Concentration:	1.0 mg/ml
Storage Buffer:	SDS-PAGE buffer (62mM Tris pH 6.8, 2% SDS, 0.9% b-mercaptoethanol, 0.003% bromophenol blue, 5% glycerol)

Description

Caspase-2 (Ich-1) belongs to the ICE/CED-3 cell death gene family of cysteine proteases. CED-3 is a *C. elegans* death gene that is required for normal programmed cell death during development. ICE (interleukin-1ß-converting enzyme) is the mammalian homolog of CED-3 and is activated when cells become apoptotic. Ich1 mRNA is alternatively spliced into two different forms, Ich-1 long (Ich-1L) and Ich-1 short (Ich-1S). Ich-1L is a 48 kDa protein that has about 50% amino acid sequence homology to both CED-3 and ICE. Ich-1S (35 kDa) is a truncated version of Ich-1L. Ich-1L and Ich-1S have been found to have opposite effects on cell death in vitro. Overexpression of Ich-1L in Rat1 rat fibroblast cells induced programmed cell death, whereas overexpression of Ich-1S suppressed Rat-1 cell death induced by serum starvation. These results suggest that Ich-1 may play a role in both the positive and negative regulation of programmed cell death.

The G310-1248 clone was made using recombinant full-length human caspase-2 (short) [Ich-1S] as the immunogen and can recognize both the short and long forms of human caspase-2.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store both the antibody and Jurkat control lysate undiluted at -20°C.

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol, Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.

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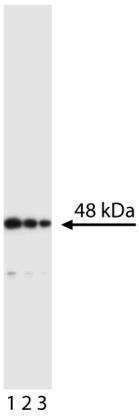
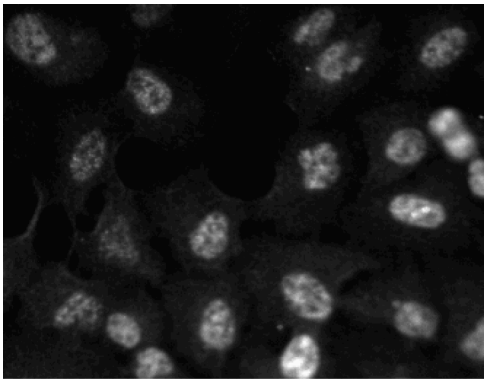
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/monoclonal_anti.jsp



Western blot analysis of Caspase-2. *Lysate from Jurkat cells were probed with the Caspase-2 antibody at concentrations of 0.5 µg/mL (lane 1), 0.25 µg/mL (lane 2), and 0.125 µg/mL (lane 3). Caspase-2 is identified as a band of 48 kDa.*



Immunofluorescent staining of U-2 OS (ATCC HTB-96) 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 alcohol perm protocol and the anti-caspase-2 antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). Images were taken on a BD Pathway™ 855 bioimaging system using a 20x objective. This antibody also stained HeLa (ATCC CCL-2) and A549 (ATCC CCL-185) cells and worked with both the Triton™ X-100 and alcohol fix/perm protocols (see Recommended Assay Procedure).*

Suggested Companion Products

Catalog Number	Name	Size	Clone
611451	Jurkat Cell Lysate	500 µg	(none)
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
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. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Triton is a trademark of the Dow Chemical Company.
5. 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.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.