

NEUROMICS

Caspase-10/b (FLICE 2) Data Sheet

Catalog Number:	RA15047	Host:	Rabbit
Product Type:	Affinity purified	Species Reactivity:	Mouse, Human
Immunogen Sequence:	KRTVWGAKQISATSLPTA(C) a.a. 487 - 504 of human Caspase 10/b (FLICE 2).	Format:	Liquid 1mg/ml 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.
Applications:	Western Blot: 1.0 µg/mL Tested in Western blot using Jurkat cells. Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. <i>Avoid repeated freeze-thaw cycles.</i>		

Application Notes

Specificity

The antibody detects human and mouse Caspase 10 precursor.

Western blotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4 0.15 M NaCl 0.1% Tween 20	5% nonfat dry milk in Blotting Buffer, Adjust pH to 7.4	5% nonfat dry milk in Blotting Buffer Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in Antibody Solution containing 0.1 µg/mL rabbit anti-Caspase-10.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a HRP-conjugated anti-rabbit IgG secondary antibody.
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with ECL Reagent (Amersham).

Cell lysates for Western blottings: To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) at 2 x 10⁶ - 1 x 10⁷ cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each. Samples are diluted with 1x SDS sample buffer to the desired concentration.

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www.neuromics.com

Neuromics Antibodies • 11200 Hampshire Avenue South • Bloomington, MN 55438
phone 507-645-8020 • fax 612-677-3976 • e-mail pshuster@neuromics1.com

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