NEUROMICS

Caspase-12 Data Sheet

Catalog Number:	RA15048	**Host:**	Rabbit
Product Type:	Affinity purified	**Species Reactivity:**	Rat, Mouse
Immunogen Sequence:	Recombinant mouse Caspase-12, amino acids 1-419 with a carboxyl-terminal six histidine tag.	**Format:**	Liquid 1mg/ml 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Applications: **Western Blot:** 0.25 µg/mL
Tested in Western blot using mouse CTLL-2 and C2C12 cells and rat PC12 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. *Avoid repeated freeze-thaw cycles.*

Application Notes

Specificity
The antibody detects endogenous rat and mouse Casapase-12 proenzyme.

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 0.25 µg/mL anti-Caspase-12.
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 blots: 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, 10 mM NaF and bromophenyl blue) at 2×10^6 - 1×10^7 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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