NEUROMICS

Calcineurin A Data Sheet

Catalog Number:	MO15055	**Host:**	Mouse
Product Type:	Protein G purified rat IgG_1 (302202).	**Species Reactivity:**	Human, Rat, Mouse
Immunogen Sequence:	Hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with purified bovine brain Calcineurin holoenzyme containing both the A and B subunits.	**Format:**	Liquid 1mg/ml Solution in phosphate-buffered saline (PBS) with 5% Trehlose
Applications:	Western Blot: 1 µg/mL		
	Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Antibody can 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 human, mouse, and rat Calcineurin A at 59 kDa in Western blots.

Western Blot

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

1. Transfer the electrophoresed proteins to Immobilon-P 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 1 µg/mL anti-human/mouse/rat Calcineurin A.
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 1:1,000 dilution of HRP-conjugated goat anti-rat IgG (Zymed).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with WesternGlo Chemiluminescent Detection Substrate or equivalent.

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, 10 mM NaF, and bromophenyl blue) at 2 x 10^7 - 1 x 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.

Description/Data:
Calcineurin A, also known as PP2B and PPP3CA, is the 59 kDa catalytic subunit of the calcium/calmodulin-dependent protein phosphatase. When activated by calcium in the presence of the regulatory B subunit and calmodulin, Calcineurin A selectively removes phosphates from serine and threonine residues on target proteins. Although ubiquitously expressed, Calcineurin levels are highest in brain, where the phosphatase plays a role in the formation and retention of memories.

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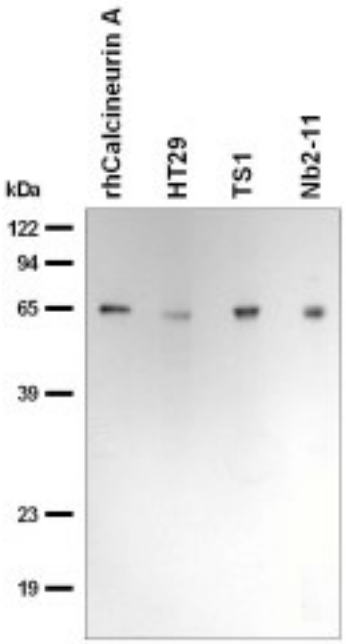
Image: Recombinant human Calcineurin A (1 ng) and lysates prepared from human HT29, mouse TS1, and rat Nb2-11 cells were resolved by SDS-PAGE, transferred to an Immobilon-P membrane, and immunoblotted with 1 µg/mL anti-Calcineurin A, as described on datasheet. A ten second exposure to film is shown.



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