



## Calcineurin A

## Data Sheet

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<b>Catalog Number:</b>	MO15055	<b>Host:</b>	Mouse
<b>Product Type:</b>	Protein G purified rat IgG <sub>1</sub> (302202).	<b>Species Reactivity:</b>	Human, Rat, Mouse
<b>Immunogen Sequence:</b>	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.	<b>Format:</b>	Liquid 1mg/ml Solution in phosphate-buffered saline (PBS) with 5% Trehlose
<b>Applications:</b>	Western Blot: 1 µg/mL  Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.		
<b>Storage:</b>	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. <i>Avoid repeated freeze-thaw cycles.</i>		

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### Application Notes

#### Specificity:

The antibody detects endogenous human, mouse, and rat Calcineurin A at 59 kDa in Western blots.

#### Western Blot

Blotting Buffer  
25 mM Tris, pH 7.4  
0.15 M NaCl  
0.1% Tween 20

Blocking Solution  
5% nonfat dry milk in Blotting Buffer  
Adjust pH to 7.4

Antibody Solution  
5% nonfat dry milk in Blotting Buffer  
Adjust pH to 7.4

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  $2 \times 10^7$  -  $1 \times 10^7$  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.

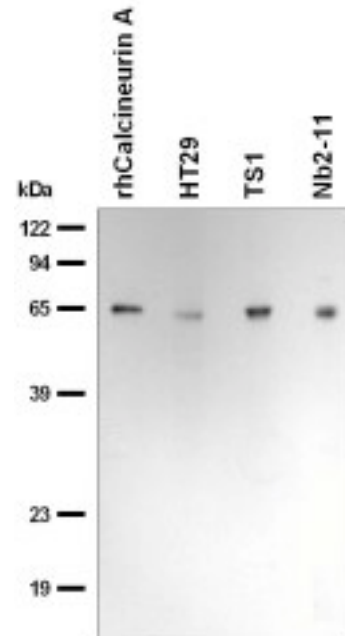
#### Description/Data:

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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