abcam

Product datasheet

Anti-Calmodulin 1/2/3 antibody [EP799Y] - C-terminal ab45689

Recombinant RabMAb



★★★★ 19 Abreviews 54 References 10 Images

Overview

Product name Anti-Calmodulin 1/2/3 antibody [EP799Y] - C-terminal

Description Rabbit monoclonal [EP799Y] to Calmodulin 1/2/3 - C-terminal

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, IHC-P, IP, IHC-Fr

Species reactivity Reacts with: Mouse, Rat, Human

Synthetic peptide within Human Calmodulin 1/2/3 aa 100 to the C-terminus. The exact sequence **Immunogen**

is proprietary.

Positive control WB: C6, RAW264.7, NIH/3T3, HeLa, HCT 116 cell lysate. IHC-P: Human urinary bladder

carcinoma and testis tissues. Flow Cyt (intra): MCF7 and NIH/3T3 cells. IP: Human skeletal

muscle tissue lysate.

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

Properties

Form

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity Tissue culture supernatant

Clonality Monoclonal
Clone number EP799Y
Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab45689 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/50 - 1/100. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB	★★★★☆ (10)	1/1000 - 1/10000. Detects a band of approximately 16 kDa (predicted molecular weight: 17 kDa).
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Mouse and rat species reactivity is recommended based on WB results, we do not guarantee IHC for mouse or rat.
IP	*** <u>*</u>	1/20 - 1/50.
IHC-Fr	★★★★ ★ <u>(2)</u>	Use at an assay dependent concentration. Mouse and rat species reactivity is recommended based on WB results, we do not guarantee IHC for mouse or rat.

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Relevance Function: Calmodulin mediates the control of a large number of enzymes and other proteins by

Ca(2+). Among the enzymes to be stimulated by the calmodulin-Ca(2+) complex are a number of protein kinases and phosphatases. Together with CEP110 and centrin, is involved in a genetic

pathway that regulates the centrosome cycle and progression through cytokinesis.

Cellular localization Cytoplasm > cytoskeleton > spindle. Cytoplasm > cytoskeleton > spindle pole. Distributed

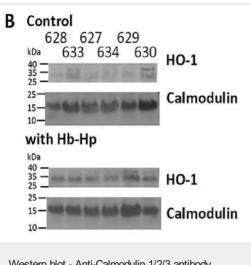
throughout the cell during interphase, but during mitosis becomes dramatically localized to the

spindle poles and the spindle microtubules.

Form There are three genes which encode an identical calcium binding protein which is one of the four

subunits of phosphorylase kinase.

Images



ΔSRCR peripheral blood monocyte-derived macrophages (PMMs) still function as hemoglobin-haptoglobin (Hb-Hp) scavengers.

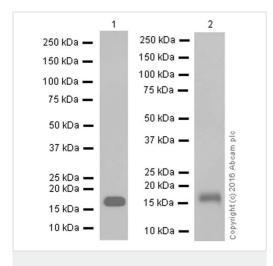
Porcine PMMs were incubated for 24 hours in the presence of 100 µg/mol Hb-Hp then lysed with reducing SDS sample buffer and HO-1 protein expression analyzed by western blot.

For full details please see paper.

Western blot - Anti-Calmodulin 1/2/3 antibody

[EP799Y] - C-terminal (ab45689)

Burkard et al PLoS Pathog. 2017 Feb 23;13(2):e1006206. doi: 10.1371/journal.ppat.1006206. eCollection 2017 Feb. Fig 8. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/



Western blot - Anti-Calmodulin 1/2/3 antibody

[EP799Y] - C-terminal (ab45689)

All lanes: Anti-Calmodulin 1/2/3 antibody [EP799Y] - C-terminal (ab45689) at 1/10000 dilution

Lane 1: HeLa (human cervix adenocarcinoma) whole cell lysate Lane 2: HCT 116 (human colorectal carcinoma) whole cell lysate

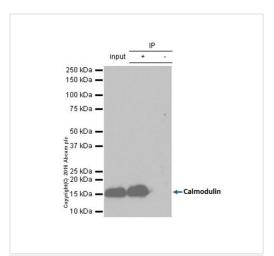
Blocking peptides at 15 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 17 kDa

Blocking and Diluting buffer: 5% NFDM/TBST



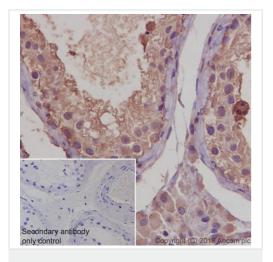
Immunoprecipitation - Anti-Calmodulin 1/2/3 antibody [EP799Y] - C-terminal (ab45689)

ab45689 immunoprecipitating Calmodulin. 10 μ g of cell lysate was incubated with primary antibody at a dilution of 1/20 and VeriBlot for IP Detection Reagent (HRP) (ab131366) at a dilution of 1/10000.

Lane 1: Human skeletal muscle lysate (10 µg)

Lane 2: Human skeletal muscle lysate

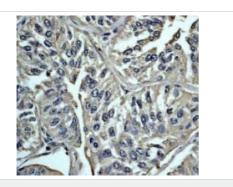
Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab45689 in human skeletal muscle lysate



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Calmodulin 1/2/3 antibody [EP799Y] - C-terminal (ab45689)

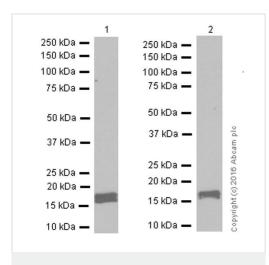
ab45689 staining Calmodulin in human testis tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/2000. A goat anti-rabbit IgG H&L (HRP) <u>ab97051</u> was used as the secondary antibody at a dilution of 1/500.

Negative control: PBS in place of primary antibody (inset).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Calmodulin 1/2/3 antibody [EP799Y] - C-terminal (ab45689)

ab45689 at a 1:250 dilution staining Calmodulin in human urinary bladder carcinoma tissue by immunohistochemistry in paraffin embedded tissue.



Western blot - Anti-Calmodulin 1/2/3 antibody [EP799Y] - C-terminal (ab45689) **All lanes :** Anti-Calmodulin 1/2/3 antibody [EP799Y] - C-terminal (ab45689) at 1/10000 dilution

Lane 1 : RAW264.7 (mouse abelson murine leukemia virusinduced tumor) whole cell lysate

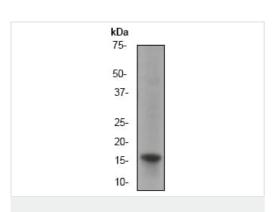
Lane 2: C6 (rat glioma) whole cell lysate

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

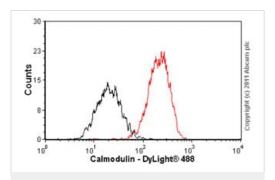
Predicted band size: 17 kDa



Western blot - Anti-Calmodulin 1/2/3 antibody [EP799Y] - C-terminal (ab45689) Blocking and diluting buffer: 5% NFDM/TBST

Anti-Calmodulin 1/2/3 antibody [EP799Y] - C-terminal (ab45689) at 1/5000 dilution + NIH/3T3 (Mouse embryo fibroblast cell line) cell lysate at 10 μ g

Predicted band size: 17 kDa **Observed band size:** 16 kDa



Flow Cytometry (Intracellular) - Anti-Calmodulin 1/2/3 antibody [EP799Y] - C-terminal (ab45689)

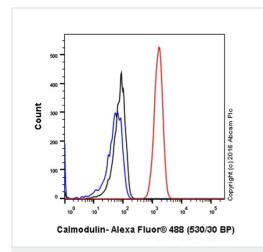
Overlay histogram showing MCF7 (Human breast adenocarcinoma cell line) cells stained with ab45689 (red line).

The cells were fixed with methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab45689, 1/50 dilution) for 30 min at 22°C. The secondary antibody used was DyLight[®] 488 goat anti-rabbit lgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C.

lsotype control antibody (black line) was rabbit monoclonal lgG (0.5µg/1x10⁶ cells) used under the same conditions.

Acquisition of >5,000 events was performed.

This antibody gave a decreased signal in MCF7 cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.



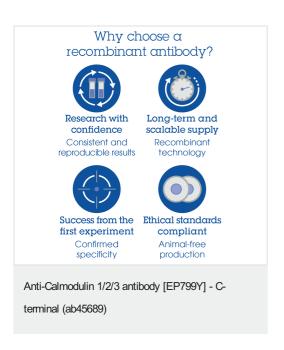
Flow Cytometry (Intracellular) - Anti-Calmodulin 1/2/3 antibody [EP799Y] - C-terminal (ab45689)

ab45689 staining Calmodulin in NIH/3T3 (Mouse embryo fibroblast cell line) cells by intracellular flow cytometry.

Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/100. A goat anti rabbit lgG (Alexa Fluorr[®] 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)

Unlabeled control: Cell without incubation with primary antibody and secondary antibody (Blue)



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