abcam

Product datasheet

Anti-Calpain small subunit 1 antibody [EPR3324] ab92333

KO VALIDATED Recombinant RabMAb

8 References 5 Images

Overview

Product name	Anti-Calpain small subunit 1 antibody [EPR3324]
Description	Rabbit monoclonal [EPR3324] to Calpain small subunit 1
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, Flow Cyt (Intra) **Unsuitable for:** IP
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat, Chinese hamster
Immunogen	Synthetic peptide within Human Calpain small subunit 1 aa 100-200. The exact sequence is proprietary.
Positive control	WB: fetal brain or fetal spleen tissue lysate; T47D or 293T cell lysate. IHC: Human kidney tissue.
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	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture supernatant
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR3324
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab92333 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
WB		
IHC-P		
Flow Cyt (Intra)		

Application notes

Flow Cyt: 1/50.
ICC: 1/100 - 1/250.
IHC-P: 1/100 - 1/250. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol. The use of an HRP/AP polymerized antibody will give a stronger signal.

WB: 1/1000 - 1/5000. Predicted molecular weight: 28 kDa.

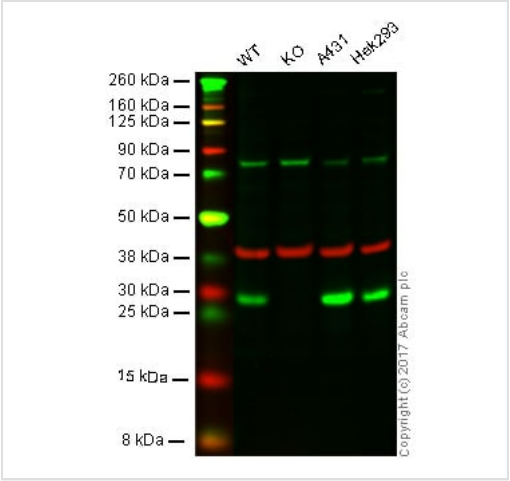
Is unsuitable for IP.

Not yet tested in other applications.
Optimal dilutions/concentrations should be determined by the end user.

Target

Function	Regulatory subunit of the calcium-regulated non-lysosomal thiol-protease which catalyzes limited proteolysis of substrates involved in cytoskeletal remodeling and signal transduction.
Sequence similarities	Contains 5 EF-hand domains.
Domain	The contact of the 5th EF-hand domain from each monomer allows the formation of the homodimer and also appears to mediate the contact between the large catalytic subunit and small regulatory subunit for the formation of the heterodimer. EF-hand domains are paired. EF-hand 1 is paired with EF-hand 2 and EF-hand 3 is paired with EF-hand 4. The fifth EF-hand domain, left unpaired, does not bind the calcium but is responsible of the dimerization by EF-embrace. The first four EF-hand domains bind calcium, however it is not sure if the binding of EF-hand 4 to calcium is physiologically relevant.
Cellular localization	Cytoplasm. Cell membrane. Translocates to the plasma membrane upon calcium binding.

Images

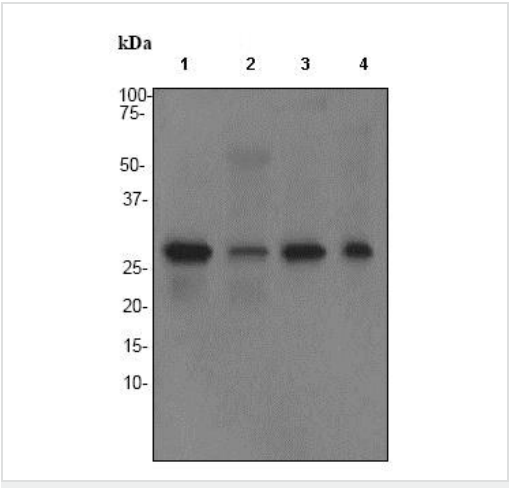


Western blot - Anti-Calpain small subunit 1 antibody [EPR3324] (ab92333)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: Calpain small subunit 1 knockout HAP1 whole cell lysate (20 µg)
Lane 3: A431 whole cell lysate (20 µg)
Lane 4: HEK293 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab92333 observed at 28 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab92333 was shown to recognize Calpain small subunit 1 when Calpain small subunit 1 knockout samples were used, along with additional cross-reactive bands. Wild-type and Calpain small subunit 1 knockout samples were subjected to SDS-PAGE. Ab92333 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Calpain small subunit 1 antibody [EPR3324] (ab92333)

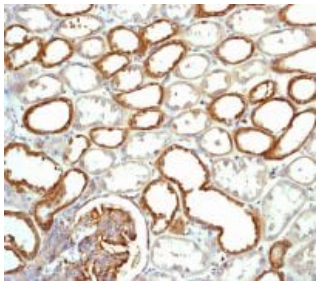
All lanes : Anti-Calpain small subunit 1 antibody [EPR3324] (ab92333) at 1/2000 dilution

Lane 1 : T-47D cell lysate
Lane 2 : Fetal brain tissue lysate
Lane 3 : Fetal spleen tissue lysate
Lane 4 : 293T (Human embryonic kidney epithelial cell) cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat anti-Rabbit HRP at 1/2000 dilution

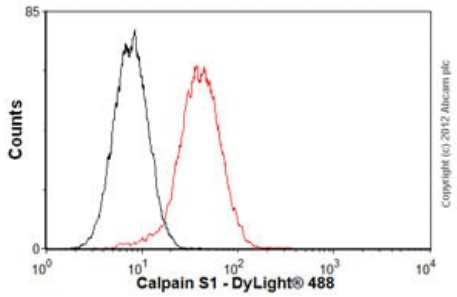
Predicted band size: 28 kDa
Observed band size: 28 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calpain small subunit 1 antibody [EPR3324] (ab92333)

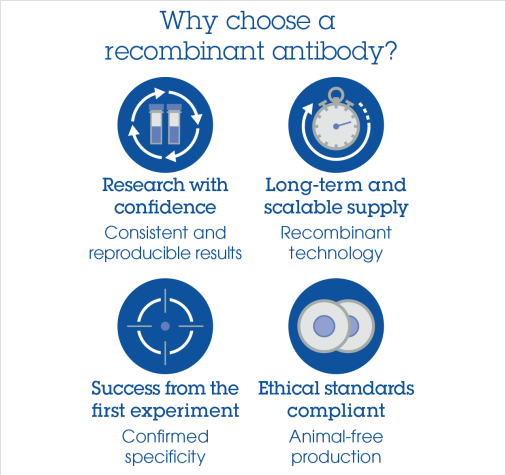
Immunohistochemistry staining of Calpain small subunit 1 in formalin-fixed, paraffin-embedded Human kidney tissue using 1/100 ab92333.

Heat mediated antigen retrieval was performed via the pressure cooker method before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-Calpain small subunit 1 antibody [EPR3324] (ab92333)

Overlay histogram showing HeLa cells stained with ab92333 (red line). The cells were fixed with 80% 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 (ab92333, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



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