abcam

Product datasheet

Anti-Calnexin antibody [EPR3633(2)] - BSA and Azide free ab225542

KO VALIDATED Recombinant RabMAb

8 Images

Overview

Product name	Anti-Calnexin antibody [EPR3633(2)] - BSA and Azide free
Description	Rabbit monoclonal [EPR3633(2)] to Calnexin - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, Flow Cyt
Species reactivity	**Reacts with:** Rat, Human
Immunogen	Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) corresponding to Human Calnexin aa 550-650 (C terminal).
Positive control	WB: WT HAP1, WT HEK-293T, HepG2, HeLa, A431, SH-SY5Y and THP1 cell lysate. IHC-P: Rat cardiac muscle; Human pancreas and kidney tissue. Flow Cyt: HeLa cells.
General notes	ab225542 is the carrier-free version of ab133615 This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. Ab225542 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm. *Maxpar® is a trademark of Fluidigm Canada Inc.* Mouse: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information. References regarding specificity: Horner SM *et al.* Mitochondrial-associated endoplasmic reticulum membranes (MAM) form innate immune synapses and are targeted by hepatitis C virus. *Proc Natl Acad Sci U S A* 108:14590-5 (2011). PubMed: 21844353 Myhill N *et al.* The subcellular distribution of calnexin is mediated by PACS-2. *Mol Biol Cell* 19:2777-88 (2008). PubMed: 18417615 Yoshimura SI *et al.* Direct targeting of cis-Golgi matrix proteins to the Golgi apparatus. *J Cell Sci* 114:4105-15 (2001). PubMed: 11739642

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

Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough.

Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.

We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee.

In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, as well as customer reviews and Q&As.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3633(2)
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab225542** 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		Use at an assay dependent concentration. Detects a band of approximately 90 kDa (predicted molecular weight: 68 kDa).

Application	Abreviews	Notes
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
Flow Cyt		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target

Function

Calcium-binding protein that interacts with newly synthesized glycoproteins in the endoplasmic reticulum. It may act in assisting protein assembly and/or in the retention within the ER of unassembled protein subunits. It seems to play a major role in the quality control apparatus of the ER by the retention of incorrectly folded proteins.

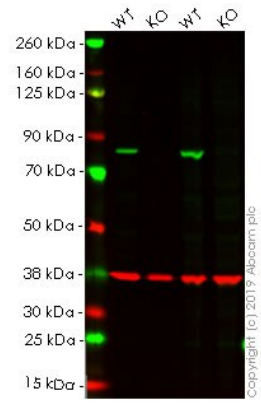
Sequence similarities

Belongs to the calreticulin family.

Cellular localization

Endoplasmic reticulum membrane. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

Images



Western blot - Anti-Calnexin antibody [EPR3633(2)] - BSA and Azide free (ab225542)

All lanes : Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615) at 1/1000 dilution

Lane 1 : Wild-type Hap1 cell lysate

Lane 2 : CANX knockout Hap1 cell lysate

Lane 3 : Wild-type HEK-293T cells cell lysate

Lane 4 : CANX knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 68 kDa

Observed band size: 90 kDa

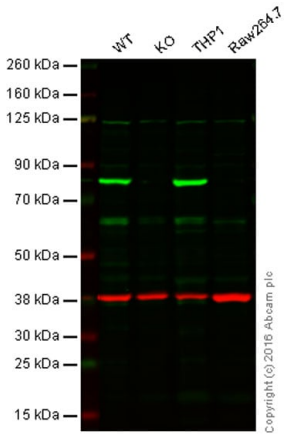
why is the actual band size different from the predicted?

This data was developed using the same antibody clone in a different buffer formulation (ab133615).

Lanes 1 - 4: Merged signal (red and green). Green - ab133615

observed at 80 kDa. Red - loading control, ab8245 observed at 37 kDa.

ab133615 was shown to react with Calnexin in wild-type HEK-293T. Loss of signal was observed when knockout cell line ab255368 (knockout cell lysate ab263805) was used. Wild-type and Calnexin knockout samples were subjected to SDS-PAGE. ab133615 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Calnexin antibody [EPR3633(2)] - BSA and Azide free (ab225542)

All lanes : Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615) at 1/1000 dilution

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : Calnexin knockout HAP1 cell lysate
Lane 3 : THP1 cell lysate
Lane 4 : RAW 264.7 cell lysate

Lysates/proteins at 20 µg per lane.

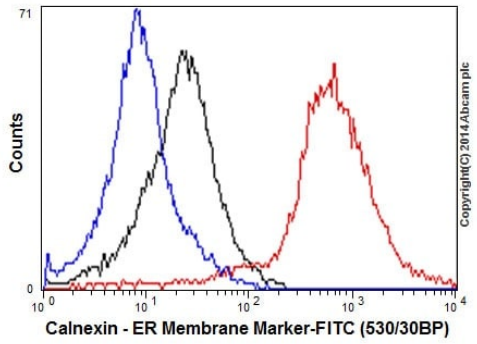
Predicted band size: 68 kDa

This WB data was generated using the same anti-Calnexin antibody clone [EPR3633(2)] in a different buffer formulation (cat# ab133615).

Lanes 1 - 4: Merged signal (red and green). Green - ab133615 observed at 80 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab133615 was shown to recognize Calnexin when Calnexin knockout samples were used, along with additional cross-reactive bands. Wild-type and Calnexin knockout samples were subjected to SDS-PAGE. ab133615 and ab8245 (loading control to GAPDH) were diluted 1/1000 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-

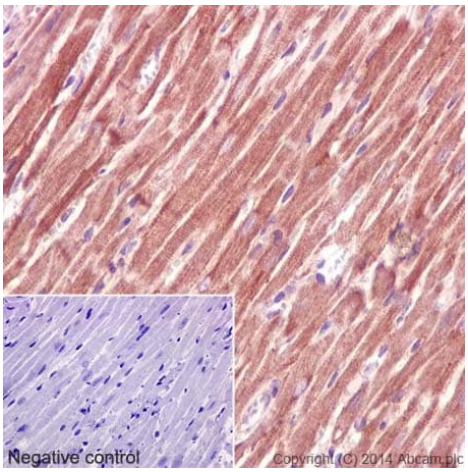
Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.



Flow Cytometry - Anti-Calnexin antibody [EPR3633(2)] - BSA and Azide free (ab225542)

Overlay histogram showing HeLa (Human epithelial cell line from cervix adenocarcinoma) cells fixed in 80% methanol and stained with purified ab133615 at a dilution of 1 in 360 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 150. Rabbit monoclonal IgG was used as an isotype control (black line) and cells without incubation with antibody were used as a negative control (blue line).

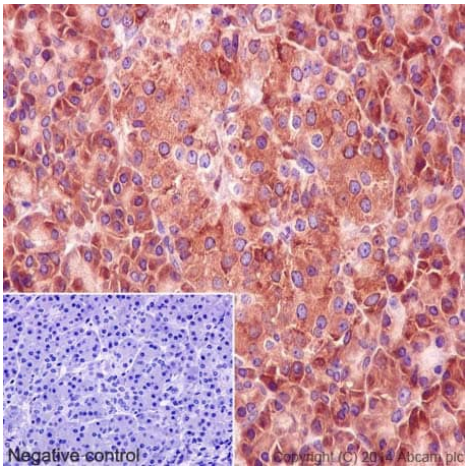
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab133615).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calnexin antibody [EPR3633(2)] - BSA and Azide free (ab225542)

Immunohistochemical staining of paraffin embedded rat cardiac muscle with purified ab133615 at a working dilution of 1 in 4000. The secondary antibody used is ab97051, a HRP goat anti-rabbit (H+L), at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

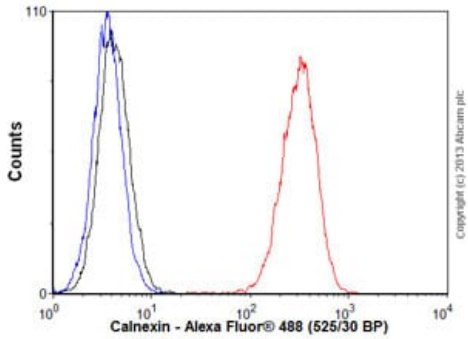
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab133615).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calnexin antibody [EPR3633(2)] - BSA and Azide free (ab225542)

Immunohistochemical staining of paraffin embedded human pancreas with purified ab133615 at a working dilution of 1 in 4000. The secondary antibody used is ab97051, a HRP goat anti-rabbit (H+L), at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

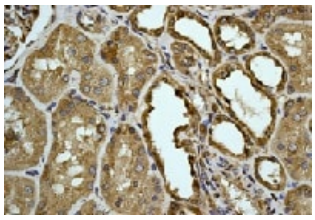
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab133615).



Flow Cytometry - Anti-Calnexin antibody [EPR3633(2)] - BSA and Azide free (ab225542)

Overlay histogram showing HeLa (Human epithelial cell line from cervix adenocarcinoma) cells stained with unpurified ab133615 (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 (unpurified ab133615, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab133615).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calnexin antibody [EPR3633(2)] - BSA and Azide free (ab225542)

Immunohistochemical analysis of paraffin-embedded human kidney tissue labelled with unpurified ab133615 at 1/100 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab133615).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Anti-Calnexin antibody [EPR3633(2)] - BSA and Azide free (ab225542)

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