abcam

Product datasheet

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

KO VALIDATED Recombinant RabMAb

7 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
Specificity	Recognizes ER membrane, mitochondria and cis-Golgi.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P
Species reactivity	**Reacts with:** Human **Does not react with:** Mouse
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: WT HAP1, WT HEK-293T, HepG2, HeLa, A431, SH-SY5Y and THP1 cell lysate. IHC-P: Human pancreas and kidney tissue. Flow Cyt (intra): HeLa cells.
General notes	ab225542 is the carrier-free version of **ab133615**. 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 **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. 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.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

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

Mouse: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

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

The Abpromise guarantee 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.

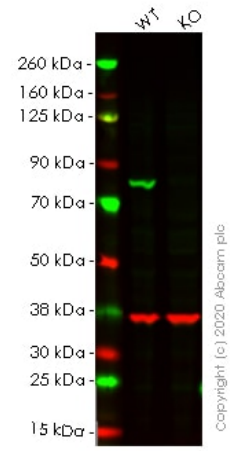
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 90 kDa (predicted molecular weight: 68 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

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 HEK-293T cell lysate
Lane 2 : CANX knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

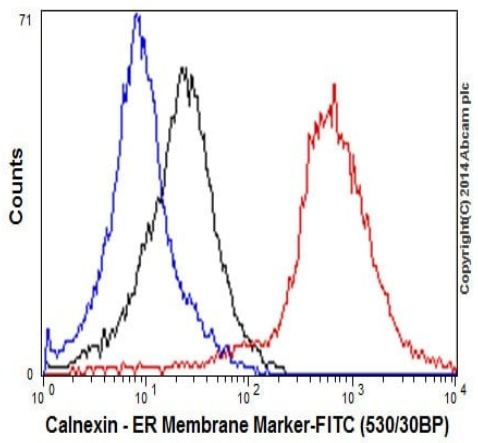
Performed under reducing conditions.

Predicted band size: 68 kDa
Observed band size: 90 kDa

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

Lanes 1- 2: Merged signal (red and green). Green - **ab133615** observed at 90 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

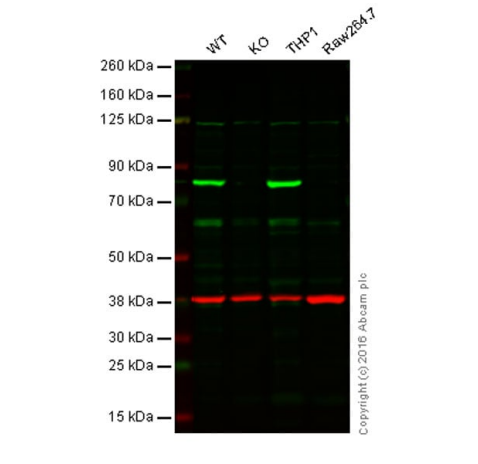
ab133615 was shown to react with Calnexin in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab255368** (knockout cell lysate **ab263805**) was used. Wild-type HEK-293T and CANX knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab133615** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 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.



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

Overlay histogram showingHeLa (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**).



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 : Raw264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) cell lysate

Lysates/proteins at 20 µg per lane.

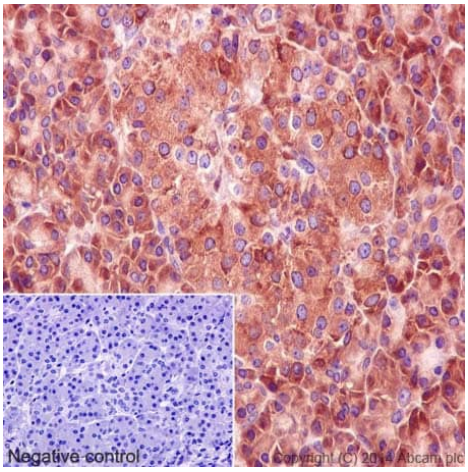
Predicted band size: 68 kDa

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

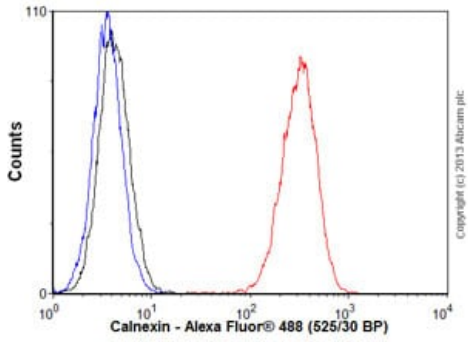
SDS-PAGE. **ab133615** and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.



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.

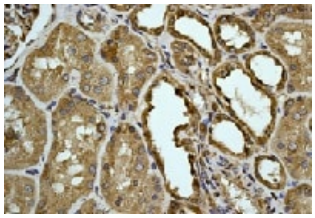
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab133615**).



Flow Cytometry (Intracellular) - 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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