abcam

Product datasheet

Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker ab133615

KO VALIDATED Recombinant RabMAb

1 Abreviews 40 References 11 Images

Overview

Product name	Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker
Description	Rabbit monoclonal [EPR3633(2)] to Calnexin - ER Membrane Marker
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 within Human Calnexin aa 550 to the C-terminus (C terminal). The exact sequence is proprietary. Database link: **P27824**
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	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** 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 +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol, PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3633(2)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab133615 in the following tested applications.

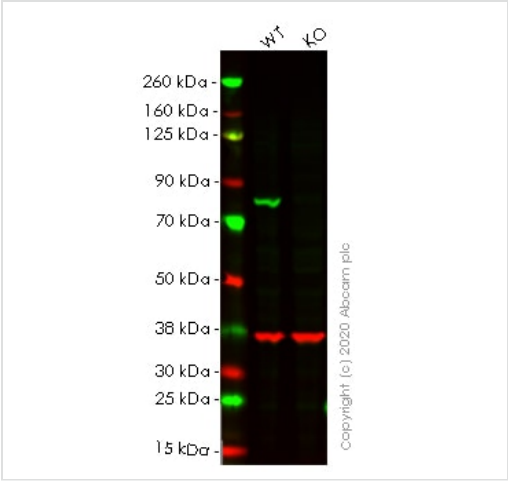
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/360. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		1/1000 - 1/5000. Detects a band of approximately 90 kDa (predicted molecular weight: 68 kDa).
IHC-P		1/4000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. **For unpurified, use 1/100 - 1/250.** 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)] - ER Membrane Marker (ab133615)

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

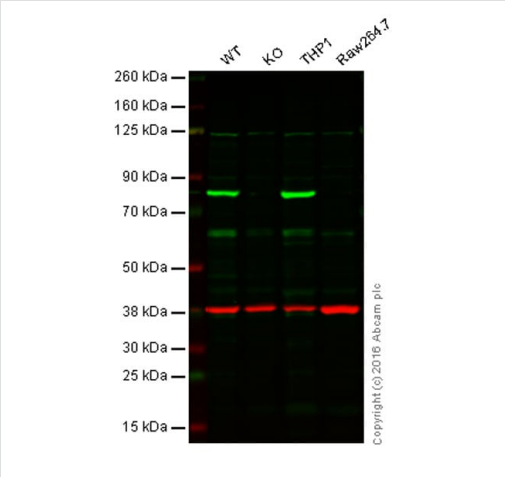
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

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.



Western blot - Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615)

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

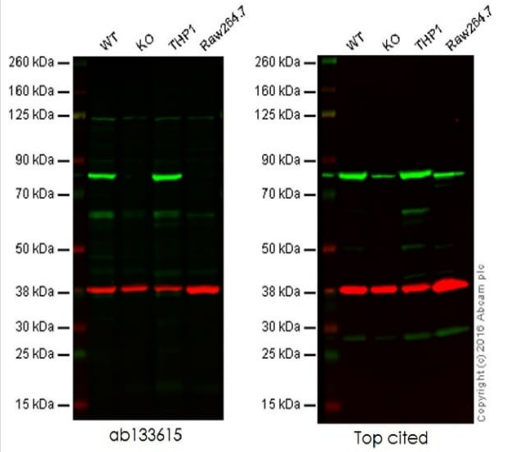
Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : Calnexin knockout HAP1 cell lysate
Lane 3 : THP1 cell lysate
Lane 4 : Raw264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 68 kDa

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.



Western blot - Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615)

All lanes : Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615)

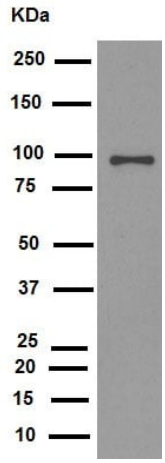
Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : Calnexin knockout HAP1 cell lysate
Lane 3 : THP1 cell lysate
Lane 4 : Raw264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 68 kDa

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

This western blot image is a comparison between ab133615 and a competitor's top cited rabbit polyclonal antibody.



Western blot - Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615)

Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615) at 1/1000 dilution (purified) + HepG2 (Human liver hepatocellular carcinoma cell line) cell lysate at 20 µg

Secondary

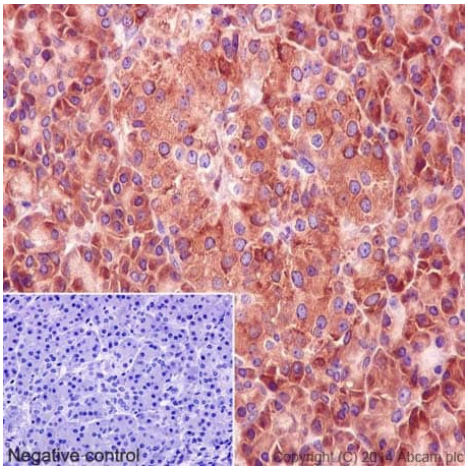
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 68 kDa

Observed band size: 90 kDa

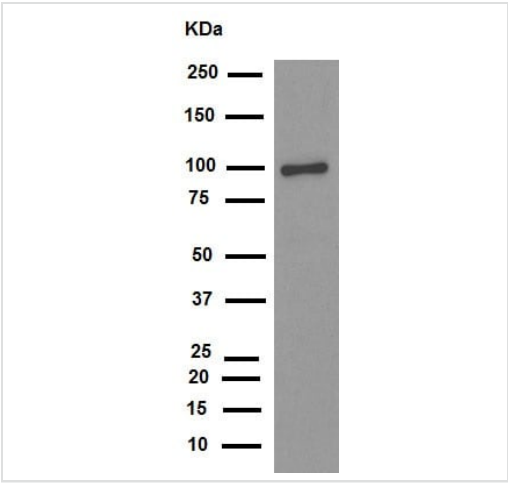
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615)

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.



Western blot - Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615)

Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615) at 1/5000 dilution (purified) + HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate at 20 µg

Secondary

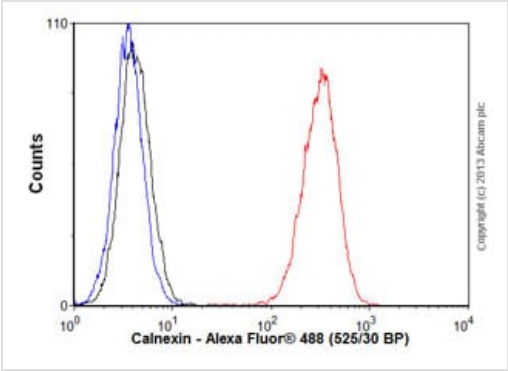
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 68 kDa

Observed band size: 90 kDa

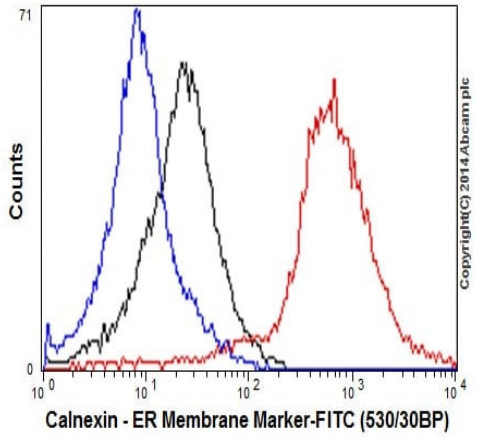
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



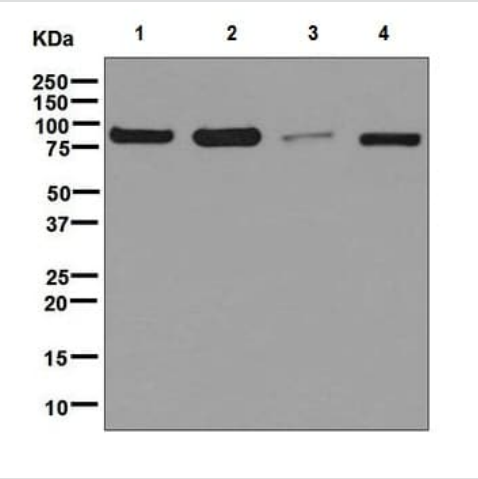
Flow Cytometry (Intracellular) - Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615)

Overlay histogram showing HeLa 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.



Flow Cytometry (Intracellular) - Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615)

Overlay histogram showingHeLa 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).



Western blot - Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615)

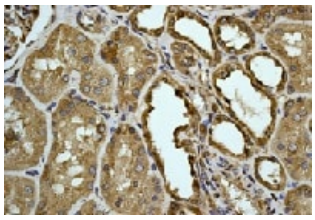
All lanes : Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615) at 1/1000 dilution (unpurified)

Lane 1 : HepG2 (Human liver hepatocellular carcinoma cell line) cell lysate
Lane 2 : A431 (Human epidermoid carcinoma cell line) cell lysate
Lane 3 : SH-SY5Y (Human neuroblastoma cell line from bone marrow) cell lysate
Lane 4 : HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615)

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

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

Anti-Calnexin antibody [EPR3633(2)] - ER Membrane Marker (ab133615)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors