abcam

Product datasheet

Anti-Calnexin antibody [EPR3632] - BSA and Azide free ab232433

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Calnexin antibody [EPR3632] - BSA and Azide free
Description	Rabbit monoclonal [EPR3632] to Calnexin - BSA and Azide free
Host species	Rabbit
Specificity	Recognizes ER membrane, mitochondria and cis-Golgi
Tested applications	**Suitable for:** WB, IP, IHC-P, ICC/IF **Unsuitable for:** Flow Cyt
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, A431, SH-SY5Y, HEK-293T, MCF7, U-2 OS and HepG2 whole cell lysate (**ab7900**). IHC-P: Human tonsil tissue. ICC/IF: Wild-type HAP1 cells. IP: HeLa lysate.
General notes	ab232433 is the carrier-free version of **ab92573**. 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. 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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3632
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab232433 in the following tested applications.

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

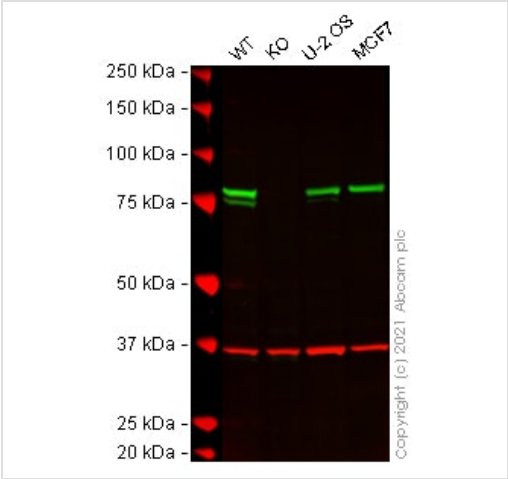
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 68 kDa.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.

Application notes Is unsuitable for Flow Cyt.

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 [EPR3632] - BSA and Azide free (ab232433)

All lanes : Anti-Calnexin antibody [EPR3632] (**ab92573**) at 1/20000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : CANX knockout HEK-293T cell lysate
Lane 3 : U-2 OS cell lysate
Lane 4 : MCF7 cell lysate

Lysates/proteins at 20 µg per lane.

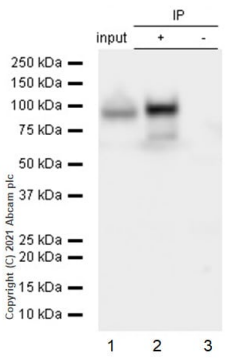
Performed under reducing conditions.

Predicted band size: 68 kDa
Observed band size: 80 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab92573**).

Lanes 1 - 4: Merged signal (red and green). Green - **ab92573** observed at 80 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab92573 was shown to react with Calnexin in wild-type HEK-293T cells in Western blot with loss of signal observed in CANX knockout cell line **ab255368** (CANX knockout cell lysate **ab263805**). Wild-type HEK-293T and CANX knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab92573** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 20000 dilution and a 1 in 20000 dilution respectively. Blots were incubated 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 in 20000 dilution for 1 h at room temperature before imaging.



Immunoprecipitation - Anti-Calnexin antibody [EPR3632] - BSA and Azide free (ab232433)

This data was developed using **ab92573**, the same antibody clone in a different buffer formulation.

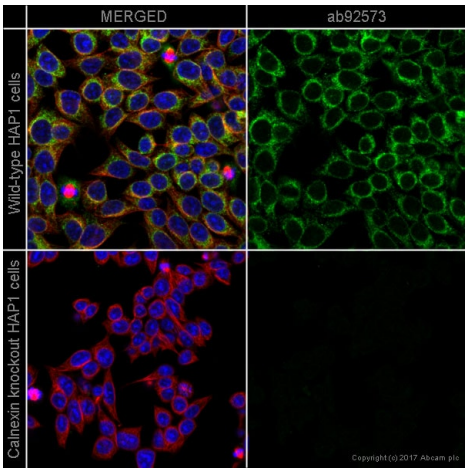
Calnexin was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg with **ab92573** at 1/100 dilution (2µg). VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg

Lane 2: abab92573 IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab92573** in HeLa whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

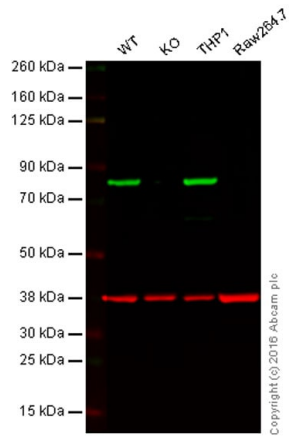


Immunocytochemistry/ Immunofluorescence - Anti-Calnexin antibody [EPR3632] - BSA and Azide free (ab232433)

ab92573 staining Calnexin in wild-type HAP1 cells (top panel) and CANX knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab92573** at 1/1000 dilution and **ab195889** at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92573**).



Western blot - Anti-Calnexin antibody [EPR3632] - BSA and Azide free (ab232433)

All lanes : Anti-Calnexin antibody [EPR3632] (**ab92573**) at 1/20000 dilution

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

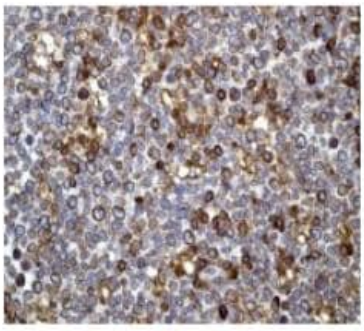
Lysates/proteins at 20 µg per lane.

Predicted band size: 68 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab92573** observed at 80 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab92573 was shown to specifically react with Calnexin when Calnexin knockout samples were used. Wild-type and Calnexin knockout samples were subjected to SDS-PAGE. **ab92573** and **ab8245** (loading control to GAPDH) were diluted at 1/20,000 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 hour at room temperature before imaging.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92573**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calnexin antibody [EPR3632] - BSA and Azide free (ab232433)

Immunohistochemical analysis of paraffin embedded Human tonsil tissue using **ab92573** at a 1/100 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92573**).

Anti-Calnexin antibody [EPR3632] - BSA and Azide free (ab232433)

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