# abcam

## Product datasheet

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





重组 RabMAb

## 1 Abreviews 1 References 6 图像

#### 概述

产品名称 Anti-Calnexin抗体[EPR3632] - BSA and Azide free

描述 兔单克隆抗体[EPR3632] to Calnexin - BSA and Azide free

宿主 Rabbit

特异性 Recognizes ER membrane, mitochondria and cis-Golgi

经测试应用 适用于: WB. IP. IHC-P. ICC/IF

不适用于: Flow Cyt

种属反应性 与反应: Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

阳性对照 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.

常规说明 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 cellbased 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<sup>®</sup> 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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

性能

形式 Liquid

**存放说明** Shipped at 4°C. Store at +4°C. Do Not Freeze.

**存储溶液** pH: 7.2

Constituent: PBS

**无载体** 是

纯**度** Protein A purified

同种型 IgG

应用

## The Abpromise guarantee Abpromise™承诺保证使用ab232433于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说 <b>明</b>
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.

应用说明 Is unsuitable for Flow Cyt.

靶标

功能 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

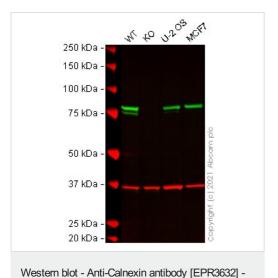
unassembled protein subunits. It seems to play a major role in the quality control apparatus of the

ER by the retention of incorrectly folded proteins.

序列相似性 Belongs to the calreticulin family.

细胞定位 Endoplasmic reticulum membrane. Melanosome. Identified by mass spectrometry in melanosome

fractions from stage I to stage  ${\sf IV}$ .



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.

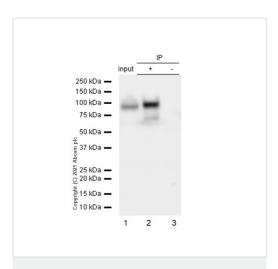
Observed band size: 80 kDa

Predicted band size: 68 kDa

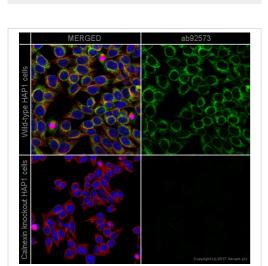
This data was developed using the same antibody clone in a different buffer formulation (ab92573).

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). Wildtype 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 lgG 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)



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

This data was developed using <u>ab92573</u>, 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  $\mu g$ 

Lane 2: abab92573 IP in HeLa whole cell lysate

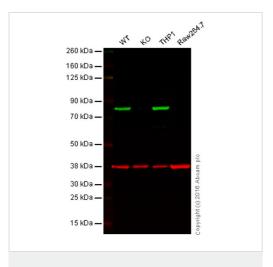
Lane 3: Rabbit monoclonal lgG ( $\underline{ab172730}$ ) instead of  $\underline{ab92573}$  in HeLa whole cell lysate

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

<u>ab92573</u> 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 <u>ab92573</u> at 1/1000 dilution and <u>ab195889</u> 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 lgG (Alexa Fluor® 488) (<u>ab150081</u>) 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 (<u>ab92573</u>).



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

**All lanes :** Anti-Calnexin antibody [EPR3632] (<u>ab92573</u>) 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

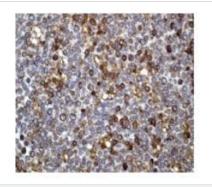
Lysates/proteins at 20 µg per lane.

Predicted band size: 68 kDa

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

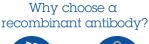


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Calnexin antibody [EPR3632] - BSA and Azide free (ab232433)

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).





Long-term and scalable supply Recombinant



specificity



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

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	7