abcam

Product datasheet

# Anti-Calnexin antibody [EPR3632] ab92573

敲除验证 重组 RabMAb

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## 概述

| | |
|---|---|
| **产品名称** | Anti-Calnexin抗体[EPR3632] |
| **描述** | 兔单克隆抗体[EPR3632] to Calnexin |
| **宿主** | Rabbit |
| **特异性** | Recognizes ER membrane, mitochondria and cis-Golgi |
| **经测试应用** | **适用于:** ICC/IF, WB, IP, IHC-P<br>**不适用于:** Flow Cyt |
| **种属反应性** | **与反应:** Human |
| **免疫原** | Synthetic peptide within Human Calnexin aa 1-100. The exact sequence is proprietary.<br>Database link: **P27824** |
| **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** | References regarding specificity:<br>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**<br>Myhill N *et al.* The subcellular distribution of calnexin is mediated by PACS-2. *Mol Biol Cell* 19:2777-88 (2008). **PubMed: 18417615**<br>Yoshimura SI *et al.* Direct targeting of cis-Golgi matrix proteins to the Golgi apparatus. *J Cell Sci* 114:4105-15 (2001). **PubMed: 11739642**<br>This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**.<br>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.<br>Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information. |

## 性能

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|---|---|
| **形式** | Liquid |
| **存放说明** | Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid repeated freeze / thaw cycles. |
| **存储溶液** | pH: 7.20<br>Preservative: 0.05% Sodium azide<br>Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture supernatant |
| **纯度** | Protein A purified |
| **克隆** | 单克隆 |
| **克隆编号** | EPR3632 |
| **同种型** | IgG |

## 应用

**The Abpromise guarantee** Abpromise™承诺保证使用ab92573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 | 说明 |
|---|---|---|
| **ICC/IF** | | 1/1000. |
| **WB** | | 1/20000 - 1/100000. Predicted molecular weight: 90 kDa. |
| **IP** | | 1/50. |
| **IHC-P** | | Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. |

**应用说明** Is unsuitable for Flow Cyt.

## 靶标

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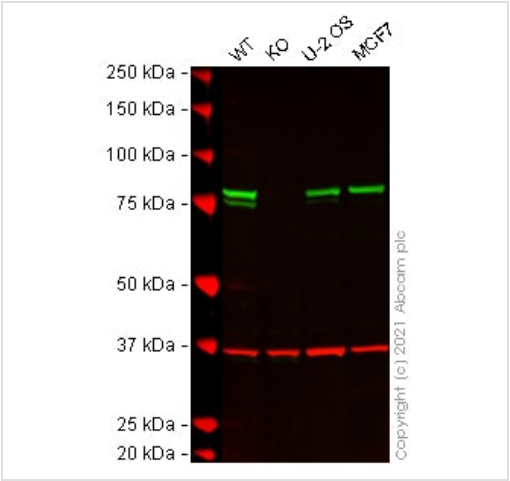
|---|---|
| **功能** | Calcium-binding protein that interacts with newly synthesized glycoproteins in the endoplasmic reticulum. It may act in assisting protein assembly and/or in the retention within the ER of unassembled protein subunits. It seems to play a major role in the quality control apparatus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

## 图片




Western blot - Anti-Calnexin antibody [EPR3632] (ab92573)

**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

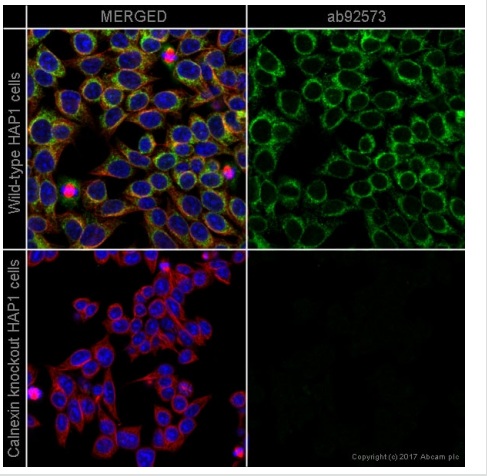
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 90 kDa
**Observed band size:** 80 kDa

**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.

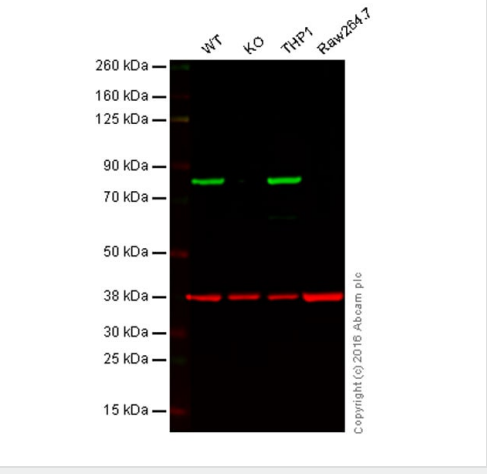
ab92573 was shown to react with Calnexin in wild-type HEK-293T cells in Western blot with loss of signal observed in CANX knockout cell line **ab255368** (CANX knockout cell lysate **ab263805**). Wild-type HEK-293T and CANX knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % 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.




Immunocytochemistry/ Immunofluorescence - Anti-Calnexin antibody [EPR3632] (ab92573)

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).




Western blot - Anti-Calnexin antibody [EPR3632] (ab92573)

**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

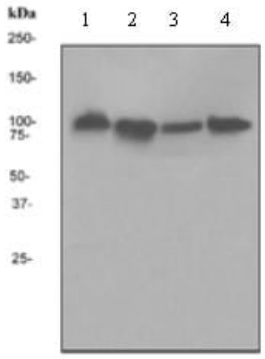
Lysates/proteins at 20 µg per lane.

**Predicted band size:** 90 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.




Western blot - Anti-Calnexin antibody [EPR3632] (ab92573)

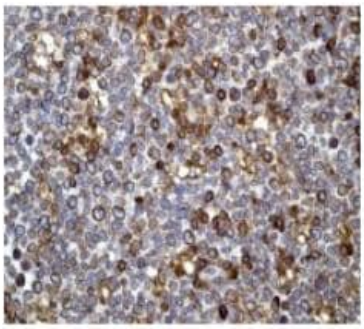
**All lanes :** Anti-Calnexin antibody [EPR3632] (ab92573) at 1/20000 dilution

**Lane 1 :** HeLa cell lysate
**Lane 2 :** A431 cell lysate
**Lane 3 :** SH-SY5Y cell lysate
**Lane 4 :** HepG2 cell lysates

Lysates/proteins at 10 µg per lane.

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

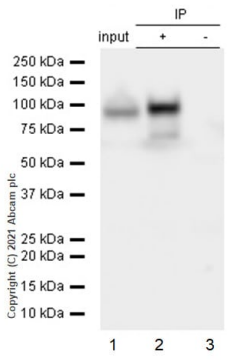
**Predicted band size:** 90 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calnexin antibody [EPR3632] (ab92573)

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.




Immunoprecipitation - Anti-Calnexin antibody [EPR3632] (ab92573)

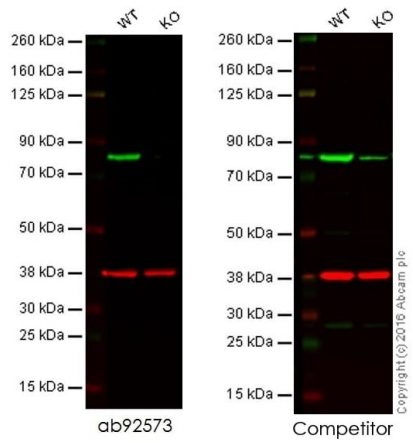
Calnexin was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg with 92573 at 1/100 dilution (2µg). VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/5000 dilution.

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

**Lane 2:** ab92573 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.




Western blot - Anti-Calnexin antibody [EPR3632] (ab92573)

**All lanes :** Anti-Calnexin antibody [EPR3632] (ab92573)

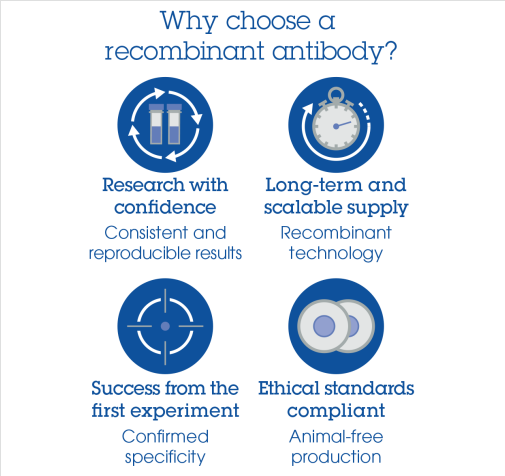
**Lane 1 :** Wild-type HAP1 cell lysate
**Lane 2 :** Calnexin knockout HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

**Predicted band size:** 90 kDa

**Lanes 1 - 2:** Merged signal (red and green). Green - ab92573 observed at 80 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

This western blot image is a comparison between ab92573 and a competitor's rabbit polyclonal antibody.




Anti-Calnexin antibody [EPR3632] (ab92573)

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