abcam

Product datasheet

Anti-Calnexin antibody - ER Marker ab22595

敲除验证

28 Abreviews 378 References 8 图像

概述

产品名称	Anti-Calnexin抗体- ER Marker
描述	兔多克隆抗体to Calnexin - ER Marker
宿主	Rabbit
特异性	Recognizes ER membrane, mitochondria and cis-Golgi
经测试应用	**适用于:** WB, ICC/IF, IP
种属反应性	**与反应:** Mouse, Rat, Human **预测可用于:** Dog, Common marmoset
免疫原	Synthetic peptide corresponding to Human Calnexin aa 550 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin. (Peptide available as **ab23379**)
常规说明	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle. Store In the Dark.
存储溶液	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

纯度	Immunogen affinity purified
克隆	**多克隆**
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用**ab22595**于以下的**经测试应用

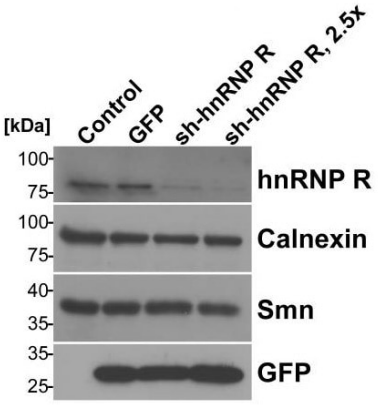
"应用说明"**部分 下显示的**仅为**推荐的起始稀释度**;实际**最佳的稀释度/浓度应由使用者检定。**

应用	Ab评论	说明
WB	★★★★★ (13)	Use a concentration of 1 µg/ml. Detects a band of approximately 75 kDa (predicted molecular weight: 90 kDa).
ICC/IF	★★★★★ (7)	Use a concentration of 1 µg/ml.
IP		Use at an assay dependent concentration.

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 IV.

图片



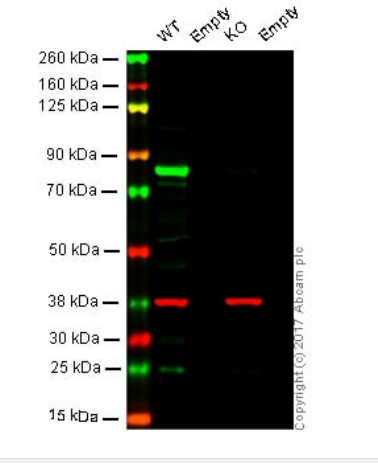
Western blot - Anti-Calnexin antibody - ER Marker (ab22595)

Dombert et al PLoS One. 2014 Oct 22;9(10):e110846. doi: 10.1371/journal.pone.0110846. eCollection 2014. Fig 1. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Subcellular distribution of Smn and hnRNP R in isolated mouse embryonic motoneurons.

Lentiviral knockdown of hnRNP R led to a dose-dependent reduction of hnRNP R levels. Calnexin and Smn protein were not altered significantly.

Primary motoneurons or E18 spinal cord tissue, respectively, were lysed with cytosolic and nuclear fractionation buffer, solubilized in Laemmli buffer and boiled for 10 minutes at 99°C. Proteins were then subjected to SDS-PAGE, blotted onto PVDF membrane, incubated with the corresponding antibodies, including ab22595.

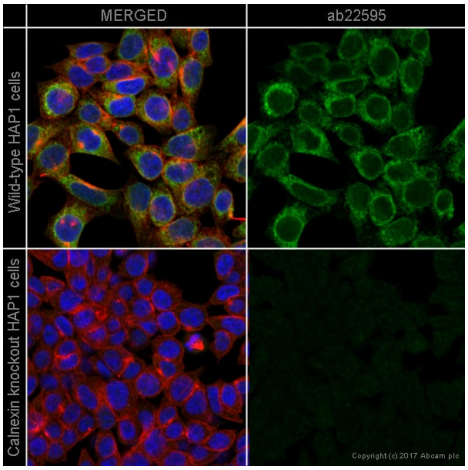


Western blot - Anti-Calnexin antibody - ER Marker (ab22595)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: empty lane
Lane 3: CANX knockout HAP1 whole cell lysate (20 µg)
Lane 4: empty lane
Lanes 1 - 4: Merged signal (red and green). Green - ab22595 observed at 80 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab22595 was shown to specifically react with CANX (Calnexin) in wildtype cells as signal was lost in CANX (Calnexin) knockout cells. Wild-type and eCANX (Calnexin) knockout samples were subjected to SDS-PAGE. ab22595 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1 dilution and 1/10,000 dilution respectively.

Blots were developed 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 1/10,000 dilution for 1 hour at room temperature before imaging.



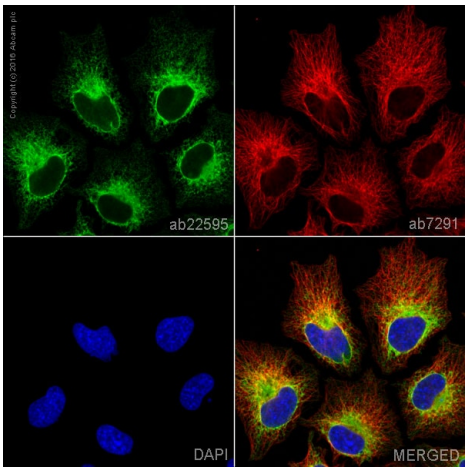
Immunocytochemistry/ Immunofluorescence - Anti-Calnexin antibody - ER Marker (ab22595)

ab22595 staining Calnexin in wild-type HAP1 cells (top panel) and CANX knockout HAP1 cells (bottom panel).

The cells were fixed with 4% formaldehyde for 10 minutes, 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 1 hour. The cells were then incubated with ab22595 at 1 µg/ml and **ab195889** at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1 hour with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green).

Nuclear DNA was labeled in blue with DAPI.

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



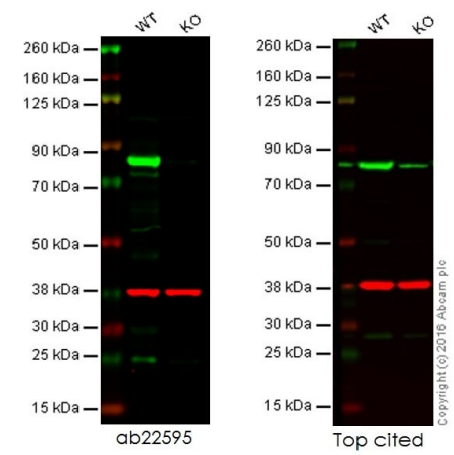
Immunocytochemistry/ Immunofluorescence - Anti-Calnexin antibody - ER Marker (ab22595)

ab22595 staining Calnexin in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells.

The cells were fixed with 100% methanol for 5 minutes, 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 1 hour. The cells were then incubated with ab22595 at 1 µg/ml and **ab7291** at 1 µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1 hour with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to Mouse IgG (Alexa Fluor® 594) (**ab150120**).

Nuclear DNA was labeled in blue with DAPI.

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

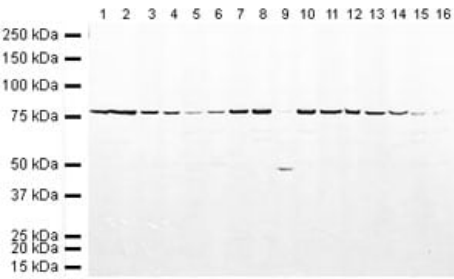


Western blot - Anti-Calnexin antibody - ER Marker (ab22595)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: Calnexin knockout HAP1 cell lysate (20 µg)

Lanes 1 - 2: Merged signal (red and green). Green - ab22595 observed at 80 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

This western blot image is a comparison between ab22595 and a competitor's top cited rabbit polyclonal antibody.



Western blot - Anti-Calnexin antibody - ER Marker (ab22595)

All lanes : Anti-Calnexin antibody - ER Marker (ab22595) at 1/250 dilution

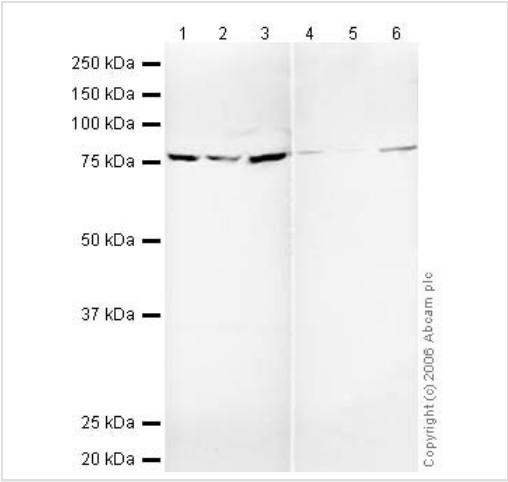
Lane 1 : NIH/3T3 whole cell lysate (**ab7179**)
Lane 2 : MEF1 (Mouse embryonic fibroblast cell line) Whole Cell Lysate (**ab46770**)
Lane 3 : Brain (Mouse) Tissue Lysate (**ab27253**)
Lane 4 : Liver (Mouse) Tissue Lysate (**ab7935**)
Lane 5 : Heart (Mouse) Tissue Lysate (**ab27255**)
Lane 6 : Kidney (Mouse) Tissue Lysate (**ab27254**)
Lane 7 : Mouse pancreas tissue lysate - total protein (**ab29363**)
Lane 8 : Testis (Mouse) Tissue Lysate - normal tissue (**ab4027**)
Lane 9 : Mouse skeletal muscle tissue lysate - total protein (**ab29711**)
Lane 10 : Spinal Cord (Mouse) Tissue Lysate (**ab50253**)
Lane 11 : Ovary (Mouse) Tissue Lysate (**ab35808**)
Lane 12 : PC-12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate (**ab50957**)
Lane 13 : Brain (Rat) Tissue Lysate (**ab7942**)
Lane 14 : Liver (Rat) Tissue Lysate (**ab27256**)
Lane 15 : Heart (Rat) Tissue Lysate (**ab7940**)
Lane 16 : Kidney (Rat) Whole Cell Lysate - normal tissue (**ab29480**)

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution

Performed under reducing conditions.

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



Western blot - Anti-Calnexin antibody - ER Marker (ab22595)

All lanes : Anti-Calnexin antibody - ER Marker (ab22595) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) whole cell lysate
Lane 2 : U-2 OS (Human bone osteosarcoma epithelial cell line) whole cell lysate
Lane 3 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate
Lane 4 : HeLa whole cell lysate with Human Calnexin peptide (**ab23379**) at 1 µg/ml
Lane 5 : U-2 OS whole cell lysate with Human Calnexin peptide (**ab23379**) at 1 µg/ml
Lane 6 : MCF7 whole cell lysate with Human Calnexin peptide (**ab23379**) at 1 µg/ml

Lysates/proteins at 20 µg per lane.

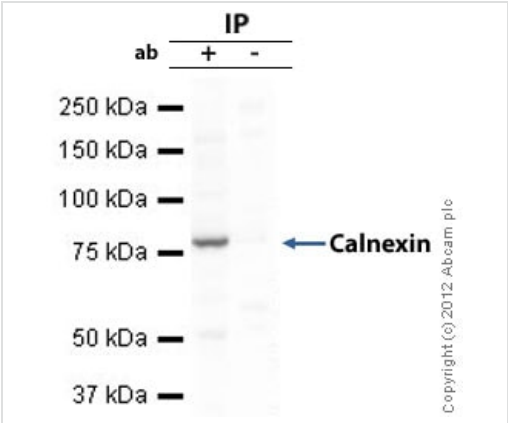
Secondary
All lanes : Goat polyclonal to Rabbit IgG (Alexa Fluor® 680) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 90 kDa
Observed band size: 75 kDa

Recent batches of ab22595 (AP217379 and AP151845) detect a band of ~ 75 kDa in HeLa, U-2 OS and MCF7 lysates. This band is completely blocked by the immunizing peptide so we believe this represents Calnexin. Moreoever, a band of the same size is

detected by other Calnexin antibodies tested.



Immunoprecipitation - Anti-Calnexin antibody - ER Marker (ab22595)

Calnexin was immunoprecipitated using 0.5 mg HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell extract, 5 µg of Rabbit polyclonal to Calnexin - ER membrane marker and 50 µl of protein G magnetic beads (+).

No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10 minutes, HeLa whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10 minutes under agitation.
Proteins were eluted by addition of 40 µl SDS loading buffer and incubated for 10 minutes at 70^{o}C; 10 µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab22595.
Secondary: Goat polyclonal to mouse IgG light chain specific (HRP) at 1/5000 dilution.
Band: 80kDa: Calnexin - ER membrane marker.

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