Overview

Product name: Anti-Calnexin antibody
Description: Rabbit polyclonal to Calnexin
Host species: Rabbit
Specificity: Recognizes ER membrane, mitochondria and cis-Golgi
Tested applications: Suitable for: WB, ICC/IF, IHC-P, IP, IHC-Fr
Species reactivity: Reacts with: Mouse, Rat, Human, Common marmoset
Predicted to work with: Dog

Immunogen: Synthetic peptide conjugated to KLH derived from within residues 550 to the C-terminus of Human Calnexin. Read Abcam's proprietary immunogen policy (Peptide available as ab23379.)
Positive control: WB: HeLa, MCF-7, NIH 3T3, MEF1 and PC12 whole cell lysates, mouse brain, liver, heart, kidney, pancreas, testis, skeletal muscle, spinal cord and ovary and rat brain, liver, heart and kidney tissue lysates. ICC/IF: HeLa and wildtype HAP1 cells.

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 or -80°C. Avoid freeze / thaw cycle. Store In the Dark.
Storage buffer: Preservative: 0.02% Sodium Azide
Constituents: 1% BSA, PBS, pH 7.4
Purity: Immunogen affinity purified
Clonality: Polyclonal
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab22595 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
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.

<table>
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<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tr>
<td>WB</td>
<td>★★★★★</td>
<td>Use a concentration of 1 µg/ml. Detects a band of approximately 75 kDa (predicted molecular weight: 90 kDa).</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>★★★★★</td>
<td>Use a concentration of 1 µg/ml.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>★★★★★</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>★★★★★</td>
<td>Use at an assay dependent concentration.</td>
</tr>
</tbody>
</table>

Target

Images
**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 1/10000 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 1/10000 dilution for 1 hour at room temperature before imaging.
ab22595 staining Calnexin in wild-type HAP1 cells (top panel) and CANX knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), 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 ab22595 at 1μg/ml 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).

ab22595 staining Calnexin in HeLa cells. 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 ab22595 at 1μg/ml and ab7291 at 1μg/ml 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) and a goat secondary antibody to Mouse IgG (Alexa Fluor® 594) (ab150120). Nuclear DNA was labelled in blue with DAPI. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).
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.

All lanes: Anti-Calnexin antibody (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: PC12 (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

Kidney cortex using ab22595 shows clear cytoplasmic staining patterns. The visceral cells of the Glomerular tuft (podocytes) are strongly stained (indicated by red arrowheads). Distal convoluted tubular cells are generally moderately positive (with exceptions that are strongly positive). However, most of the cells that line the Proximal Convoluted Tubules (indicated by green arrowheads) are strongly positive.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calnexin antibody (ab22595)

This image is courtesy of an abreview submitted by Carl Hobbs, King's College London, United Kingdom
**All lanes**: Anti-Calnexin antibody (ab22595) at 1 µg/ml

**Lane 1**: HeLa (Human epithelial carcinoma cell line)

**Lane 2**: U2OS Whole Cell Lysate

**Lane 3**: MCF-7 (Human breast adenocarcinoma cell line) Whole Cell Lysate

**Lane 4**: HeLa (Human epithelial carcinoma cell line) with Human Calnexin peptide (ab23379) at 1 µg/ml

**Lane 5**: U2OS Whole Cell Lysate with Human Calnexin peptide (ab23379) at 1 µg/ml

**Lane 6**: MCF-7 (Human breast adenocarcinoma cell line) 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, U2OS and MCF-7 lysates. This band is completely blocked by the immunizing peptide so we believe this represents Calnexin. Moreover, a band of the same size is detected by other Calnexin antibodies tested.
Calnexin - ER membrane marker was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Rabbit polyclonal to Calnexin - ER membrane marker and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation. Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl 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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