Anti-Calreticulin antibody [EPR3924] - ER Marker
ab92516

Overview

Product name: Anti-Calreticulin antibody [EPR3924] - ER Marker
Description: Rabbit monoclonal [EPR3924] to Calreticulin - ER Marker
Host species: Rabbit
Tested applications: Suitable for: WB, IHC-P, Flow Cyt, ICC/IF
Species reactivity: Reacts with: Mouse, Rat, Human, Monkey
Immunogen: Synthetic peptide within Human Calreticulin aa 50-150. The exact sequence is proprietary. (Peptide available as ab180826)
Positive control: WB: SH-SY5Y, HL-60, HepG2, HeLa, Fetal kidney and Fetal brain lysates; Human kidney tissue; Mouse and Rat brain lysates. ICC/IF: HAP1 cells (HAP1-CALR as negative cell line) IHC-P: Human colon, kidney, liver, placenta, stomach, breast carcinoma and Papillary carcinoma of thyroid gland tissues; Mouse liver and Rat lung tissues.
General notes: 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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer: pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: 9% PBS, 40% Glycerol, 0.05% BSA, 50% Tissue culture supernatant
### Purity
Protein A purified

### Clonality
Monoclonal

### Clone number
EPR3924

### Isotype
IgG

### Applications

Our **Abpromise guarantee** covers the use of ab92516 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<td>IHC-P</td>
<td>1/250 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. The use of a HRP/AP polymerized secondary antibody is recommended for enhanced staining.</td>
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<tr>
<td>Flow Cyt</td>
<td>1/10 - 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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<tr>
<td>ICC/IF</td>
<td>1/500.</td>
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### Function
Molecular calcium-binding chaperone promoting folding, oligomeric assembly and quality control in the ER via the calreticulin/calnexin cycle. This lectin interacts transiently with almost all of the monoglucosylated glycoproteins that are synthesized in the ER. Interacts with the DNA-binding domain of NR3C1 and mediates its nuclear export.

### Sequence similarities
Belongs to the calreticulin family.

### Domain
Can be divided into a N-terminal globular domain, a proline-rich P-domain forming an elongated arm-like structure and a C-terminal acidic domain. The P-domain binds one molecule of calcium with high affinity, whereas the acidic C-domain binds multiple calcium ions with low affinity. The interaction with glycans occurs through a binding site in the globular lectin domain. The zinc binding sites are localized to the N-domain. Associates with PDIA3 through the tip of the extended arm formed by the P-domain.

### Cellular localization
Endoplasmic reticulum lumen. Cytoplasm > cytosol. Secreted > extracellular space > extracellular matrix. Cell surface. Also found in cell surface (T cells), cytosol and extracellular matrix. Associated with the lytic granules in the cytolytic T-lymphocytes.

### Images

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**Lane 1:** Wild-type HAP1 cell lysate (20 µg)

**Lane 2:** Calreticulin knockout HAP1 cell lysate (20 µg)

**Lane 3:** HeLa cell lysate (20 µg)

**Lane 4:** NIH3T3 cell lysate (20 µg)

**Lanes 1 - 4:** Merged signal (red and green). Green - ab92516 observed at 55 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab92516 was shown to specifically react with Calreticulin when Calreticulin knockout samples were used. Wild-type and Calreticulin knockout samples were subjected to SDS-PAGE. ab92516 and ab8245 (loading control to GAPDH) were diluted 1/1000 and 1/10000 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 1/10000 dilution for 1 hour at room temperature before imaging.

**Immunocytochemistry/ Immunofluorescence - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)**

ab92516 staining Calreticulin in wild-type HAP1 cells (top panel) and CALR 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 ab92516 at 1/500 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).

Alexa Fluor® 488 (ab196158) and Alexa Fluor® 647 (ab196159) conjugated versions are available for this clone.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516)

Immunohistochemical analysis of paraffin-embedded Rat lung tissue labeling Calreticulin with ab92516, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic staining on rat lung. The section was incubated with ab229902 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

All lanes: Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516) at 1/10000 dilution

Lane 1: HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates
Lane 2: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 3: Mouse brain lysates
Lane 4: Rat brain lysates
Lane 5: COS-1 (African green monkey kidney fibroblast-like) whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 48 kDa
Observed band size: 55 kDa why is the actual band size different from the predicted?
Blocking/Diluting buffer and concentration: 5% NFDM/TBST
Exposure time: Lane 1 to 3: 10 seconds; Lane 4 and 5: 130 seconds

Overlay histogram showing HAP1 wildtype (green line) and HAP1-CALR knockout cells (red line) stained with ab92516. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab92516, 1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) preadsorbed (ab150081) at 1/2000 dilution for 30 min at 22°C.

A rabbit IgG isotype control antibody (ab172730) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-CALR knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.

Alexa Fluor® 488 (ab196158) and Alexa Fluor® 647 (ab196159) conjugated versions are available for this clone.

Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling Calreticulin with ab92516, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic staining on mouse liver. The section was incubated with ab229902 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).
Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labeling Calreticulin with ab92516, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic staining human breast carcinoma. The section was incubated with ab229902 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Formalin-fixed, paraffin-embedded normal human colon tissue stained for Calreticulin using ab92516 at 1/250 dilution in immunohistochemical analysis.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
Immunocytochemistry/Immunofluorescence analysis of HT-29 (human colorectal adenocarcinoma) labelling Calreticulin with purified ab92516 at 1/500. Cells were fixed with 100% methanol. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (Ab150077). Nuclei counterstained with DAPI (blue).

Control: PBS only

Overlay histogram showing HeLa cells stained with ab92516 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab92516, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

**All lanes** : Anti-Calreticulin antibody [EPR3924] - ER Marker (ab92516) at 1/1000 dilution

**Lane 1** : SH-SY5Y cell lysate
**Lane 2** : HL-60 cell lysate
**Lane 3** : HepG2 cell lysate
**Lane 4** : HeLa cell lysate
**Lane 5** : Human fetal kidney lysate
**Lane 6** : Human fetal brain lysate

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes** : HRP labelled goat anti-rabbit at 1/2000 dilution
**Predicted band size:** 48 kDa

ab92516, at 1/250 dilution, staining Calreticulin in paraffin embedded Human kidney tissue by Immunohistochemistry.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

ab92516 showing negative staining in Normal human heart tissue.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Formalin-fixed, paraffin-embedded normal human liver tissue stained for Calreticulin using ab92516 at 1/250 dilution in immunohistochemical analysis.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
Formalin-fixed, paraffin-embedded normal human placenta tissue stained for Calreticulin using ab92516 at 1/250 dilution in immunohistochemical analysis.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Formalin-fixed, paraffin-embedded normal human stomach tissue stained for Calreticulin using ab92516 at 1/250 dilution in immunohistochemical analysis.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Formalin-fixed, paraffin-embedded Papillary carcinoma of human thyroid gland tissue stained for Calreticulin using ab92516 at 1/250 dilution in immunohistochemical analysis.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

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