Overview

Product name: Anti-Calreticulin antibody [EPR3924] - ER Marker (HRP)
Description: Rabbit monoclonal [EPR3924] to Calreticulin - ER Marker (HRP)
Host species: Rabbit
Conjugation: HRP
Tested applications: Suitable for: WB, IHC-P
Species reactivity: Reacts with: Human
Predicted to work with: Mouse, Rat
Immunogen: Synthetic peptide within Human Calreticulin aa 50-150. The exact sequence is proprietary. (Peptide available as ab180826)
Positive control: WB: HepG2 and HeLa whole cell lysates. Human Fetal Brain tissue lysate. IHC-P: FFPE human normal kidney tissue sections.
General notes: Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents. This product is a recombinant rabbit monoclonal antibody.

Properties

Form: Liquid
Storage buffer: pH: 7.4
Preservative: 0.1% Proclin
Constituents: PBS, 30% Glycerol, 1% BSA
Purity: Protein A purified
Clonality: Monoclonal
Clone number: EPR3924
Isotype: IgG
Applications

Our Abpromise guarantee covers the use of ab195511 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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<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<td>WB</td>
<td>1/5000. Detects a band of approximately 55 kDa (predicted molecular weight: 48 kDa).</td>
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<tr>
<td>IHC-P</td>
<td>1/250 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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Target

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
All lanes: Anti-Calreticulin antibody [EPR3924] - ER Marker (HRP) (ab195511) at 1/5000 dilution

Lane 1: Wild-type HAP1 whole cell lysate
Lane 2: CALR knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 48 kDa
Observed band size: 55 kDa

why is the actual band size different from the predicted?

Exposure time: 90 seconds

ab195511 was shown to recognise Calreticulin in wild-type HAP1 cells as signal was lost at the expected MW in CALR knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and CALR knockout samples were subjected to SDS-PAGE. Ab195511 was incubated overnight at 4°C at 1/5000 dilution. Blots were developed with ECL technique.

IHC image of Calreticulin staining in a section of formalin-fixed paraffin-embedded human normal kidney*. The section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH6) for 30mins, and incubated overnight at +4°C with ab195511 at 1µg/ml. DAB was used as the chromogen (ab103723), diluted 1/100 and incubated for 10min at room temperature. The section was counterstained with haematoxylin and mounted with DPX. The inset negative control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre
All lanes: Anti-Calreticulin antibody [EPR3924] - ER Marker (HRP) (ab195511) at 1/5000 dilution

Lane 1: HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate
Lane 2: HeLa whole cell lysate (ab150035)
Lane 3: Brain (Human) Tissue Lysate - fetal normal tissue

Lysates/proteins at 10 µg per lane.

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 48 kDa

Observed band size: 55 kDa

*why is the actual band size different from the predicted?*

Exposure time: 8 seconds

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab195511 overnight at 4°C. Antibody binding was visualised using ECL development solution ab133406.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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