Product datasheet

Anti-Cathepsin D antibody ab72915

Overview

Product name                  Anti-Cathepsin D antibody
Description                  Rabbit polyclonal to Cathepsin D
Host species                 Rabbit
Tested applications          Suitable for: WB, ICC/IF, IHC-P
Species reactivity           Reacts with: Human
Predicted to work with       Dog, Pig, Orangutan
Immunogen                    Synthetic peptide conjugated to KLH derived from within residues 200 - 300 of Human Cathepsin D. Read Abcam's proprietary immunogen policy (Peptide available as ab90605.)
Positive control             WB: MCF7, A431 and HepG2 whole cell lysates. ICC/IF: methanol fixed HepG2 cells. IHC-P: human adrenal gland tissue.

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.
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 ab72915 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>★★★★★</td>
<td>Use a concentration of 1 µg/ml. Detects a band of approximately 45 kDa (predicted molecular weight: 45 kDa).</td>
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</tbody>
</table>
Function
Acid protease active in intracellular protein breakdown. Involved in the pathogenesis of several
diseases such as breast cancer and possibly Alzheimer disease.

Tissue specificity
Expressed in the aorta extracellular space (at protein level).

Involvement in disease
Ceroid lipofuscinosis, neuronal, 10

Sequence similarities
Belongs to the peptidase A1 family.
Contains 1 peptidase A1 domain.

Post-translational modifications
N- and O-glycosylated.

Cellular localization
Lysosome. Melanosome. Secreted, extracellular space. Identified by mass spectrometry in
melanosome fractions from stage I to stage IV. In aortic samples, detected as an extracellular
protein loosely bound to the matrix (PubMed:20551380).

Images

**Application | Abreviews | Notes**

<table>
<thead>
<tr>
<th>ICC/IF</th>
<th>Use a concentration of 5 µg/ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC-P</td>
<td>Use a concentration of 0.1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
</tr>
</tbody>
</table>

**Target**

**Function**
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**Images**

**All lanes** : Anti-Cathepsin D antibody (ab72915) at 1 µg/ml

**Lane 1** : MCF7 (Human breast adenocarcinoma cell line) Whole Cell Lysate

**Lane 2** : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate

**Lane 3** : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes** : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

**Predicted band size:** 45 kDa

**Observed band size:** 29, 45, 48 kDa

**why is the actual band size different from the predicted?**
Additional bands at: 150 kDa, 90 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 4 minutes

Cathepsin D has a predicted molecular weight of 45 kDa. The sequence contains a signal sequence and propeptide of 18 and 45 amino acids, respectively. This protein is further cleaved to produce a heavy and light chain with molecular weights of 27 kDa and 11 kDa, respectively (SwissProt). We hypothesize that the observed bands at 29 kDa represent the Cathepsin heavy chain, and the bands at 45 and 48 kDa represent the protein with and without the presence of the signal peptide.

IHC image of Cathepsin D staining in human adrenal gland formalin fixed paraffin embedded tissue section, performed on a Leica Bond system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab72915, 0.1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

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.

ICC/IF image of ab72915 stained HepG2 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Triton for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab72915 at 5µg/ml and ab7291 (Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control) at 1/1000 dilution overnight at +4°C. and ab7291 (Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control) at 1/1000 dilution overnight at +4°C. The secondary antibodies were ab150120 (pseudo-colored red) and ab150081 (colored green) used at 1 µg/ml for 1 hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43 µM for 1 hour at room temperature.
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