**Product datasheet**

**Anti-Cathepsin D antibody [EPR3057Y] ab75852**

**Overview**

**Product name**  
Anti-Cathepsin D antibody [EPR3057Y]

**Description**  
Rabbit monoclonal [EPR3057Y] to Cathepsin D

**Host species**  
Rabbit

**Tested applications**  
Suitable for: Flow Cyt, ICC/IF, WB, IP, IHC-P

**Species reactivity**  
Reacts with: Mouse, Human

**Immunogen**  
Synthetic peptide within Human Cathepsin D aa 350 to the C-terminus (C terminal). The exact sequence is proprietary.

**Positive control**  

**General notes**  
Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

**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: PBS, 40% Glycerol, 0.05% BSA

**Purity**  
Protein A purified
Clonality: Monoclonal
Clone number: EPR3057Y
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab75852 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>Abreviews</th>
<th>Notes</th>
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<td>Flow Cyt</td>
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<td>Use at an assay dependent concentration.</td>
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<tr>
<td>ICC/IF</td>
<td>1/100 - 1/1000.</td>
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<td>IP</td>
<td>1/10 - 1/20.</td>
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<tr>
<td>IHC-P</td>
<td>1/100 - 1/1000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.</td>
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Target

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
All lanes: Anti-Cathepsin D antibody [EPR3057Y] (ab75852) at 1/5000 dilution (purified)

Lane 1: MCF7 (Human breast adenocarcinoma cell line) whole cell lysate
Lane 2: SK-BR-3 (Human mammary gland adenocarcinoma cell line) whole cell lysate
Lane 3: HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/10000 dilution

Predicted band size: 46 kDa
Observed band size: 28, 43, 46 kDa

why is the actual band size different from the predicted?

Blocking/Dilution buffer: 5% NFDM /TBST.
Immunocytochemistry/Immunofluorescence analysis of MCF7 (Human breast adenocarcinoma cell line) cells labeling Cathepsin D with purified ab75852 at 1/100.

Cells were fixed with 100% methanol and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/1000) and ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.

**Control 1:** primary antibody (1/100) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

**Control 2:** ab7291 (1/1000) and secondary antibody, ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labeling Cathepsin D with purified ab75852 at a dilution of 1/100.

Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, an HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500).

**Negative control** using PBS instead of primary antibody. Counterstained with hematoxylin.
Anti-Cathepsin D antibody [EPR3057Y] (ab75852) at 1/5000 dilution (purified) + Mouse brain tissue lysate at 20 µg

**Secondary**

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/10000 dilution

**Predicted band size:** 46 kDa  
**Observed band size:** 28,43 kDa  
*why is the actual band size different from the predicted?*

**Blocking/Dilution buffer:** 5% NFDM /TBST.

Flow Cytometry analysis of HepG2 (Human liver hepatocellular carcinoma cell line) cells labeling Cathepsin D with purified ab75852 at 1/20 dilution (10 µg/ml) (red).

Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488)(1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.

ab75852 (purified) at 1/20 immunoprecipitating Cathepsin D in SK-BR-3 (Human mammary gland adenocarcinoma cell line) whole cell lysate.

**Lane 1** (input): SK-BR-3 whole cell lysate (10µg)

**Lane 2** (+): ab75852 + SK-BR-3 whole cell lysate (10µg).

**Lane 3** (-): Rabbit monoclonal IgG (ab172730) instead of ab75852 in SK-BR-3 whole cell lysate.

For western blotting, ab131366 VeriBlot for IP (HRP) was used as the secondary antibody (1/10,000).

**Blocking/Dilution buffer and concentration:** 5% NFDM/TBST.
**Western blot - Anti-Cathepsin D antibody [EPR3057Y] (ab75852)**

**All lanes** : Anti-Cathepsin D antibody [EPR3057Y] (ab75852) at 1/10000 dilution (unpurified)

**Lane 1** : MCF7 (Human breast adenocarcinoma cell line) cell lysate

**Lane 2** : A431 (Human epidermoid carcinoma cell line) cell lysate

**Lane 3** : SK-BR-3 (Human mammary gland adenocarcinoma cell line) cell lysate

**Lane 4** : HepG2 (Human liver hepatocellular carcinoma cell line) cell lysate

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes** : HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

**Predicted band size** : 46 kDa

**Observed band size** : 46 kDa

**Additional bands at** : 28 kDa (possible cleavage fragment)

Bands at 28kDa are the Cathepsin D heavy chain.

**Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cathepsin D antibody [EPR3057Y] (ab75852)**

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labeling Cathepsin D with unpurified ab75852 at a dilution of 1/500.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labeling Cathepsin D with unpurified ab75852 at a dilution of 1/500.

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