abcam

Product datasheet

Anti-Cathepsin D antibody [EPR3057Y] ab75852

Recombinant RabMAb

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Overview

Product name	Anti-Cathepsin D antibody [EPR3057Y]
Description	Rabbit monoclonal [EPR3057Y] to Cathepsin D
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), ICC/IF, WB, IP, IHC-P, IHC-Fr
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	WB: MCF7, A431, SK-BR-3 and HepG2 whole cell lysate (ab7900) and mouse brain tissue lysate. IHC-P: Human breast carcinoma and liver tissues. ICC/IF: MCF7 cells. IP: SK-BR-3 cell lysate. Flow Cyt (intra): HepG2 cells. IHC-Fr: Hu liver tissue sections.
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 <u>see here</u>. Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u>. Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

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
lsotype	lgG

Applications

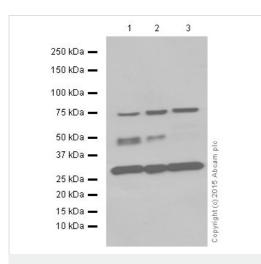
The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab75852 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF	★ ★ ★ ★ ★ <u>(1)</u>	1/100 - 1/1000.
WB	★ ★ ★ ★ ★ <u>(8)</u>	1/2000 - 1/10000. Predicted molecular weight: 46 kDa.
IP		1/10 - 1/20.
IHC-P	★ ★ ★ ★ ★ (1)	1/100 - 1/1000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols .
IHC-Fr	★★★★ <u></u> (1)	1/1000.

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 extrcellular 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



Western blot - Anti-Cathepsin D antibody [EPR3057Y] (ab75852)

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

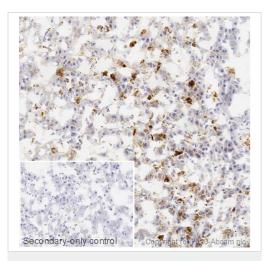
Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/10000 dilution

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

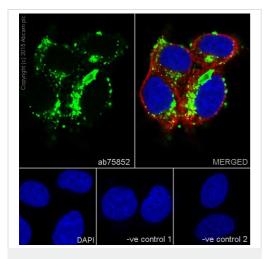
Blocking/Dilution buffer: 5% NFDM /TBST.



Immunohistochemistry (Frozen sections) - Anti-Cathepsin D antibody [EPR3057Y] (ab75852)

IHC image of Cathepsin D staining in a section of frozen normal human liver performed on a Leica BONDTM system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab75852, 1/1000 dilution, 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. The inset secondary-only 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.



Immunocytochemistry/ Immunofluorescence - Anti-Cathepsin D antibody [EPR3057Y] (ab75852)

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 antirabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse antitubulin (1/1000) and **ab150120**, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/1000) were also used.

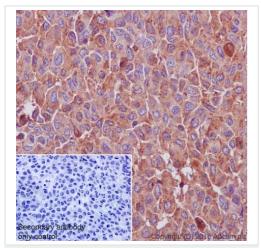
Control 1: primary antibody (1/100) and secondary antibody, <u>**ab150120**</u>, an Alexa Fluor[®] 594-conjugated goat anti-mouse lgG (1/1000).

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

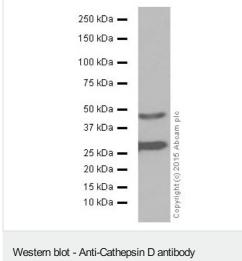
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labeling Cathepsin D with purified ab75852 at a dilution of 1/100.

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

Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



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



[EPR3057Y] (ab75852)



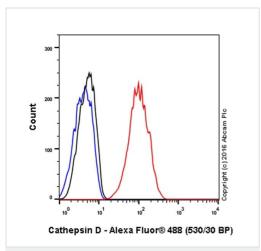
Anti-Cathepsin D antibody [EPR3057Y] (ab75852) at 1/5000

dilution (purified) + Mouse brain tissue lysate at 20 µg

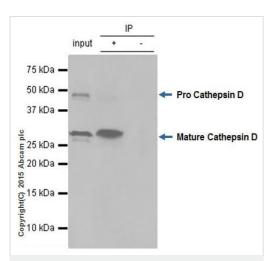
Observed band size: 28,43 kDa

Secondary

Blocking/Dilution buffer: 5% NFDM /TBST.



Flow Cytometry (Intracellular) - Anti-Cathepsin D antibody [EPR3057Y] (ab75852)



Immunoprecipitation - Anti-Cathepsin D antibody [EPR3057Y] (ab75852) Intracellular Flow Cytometry analysis of HepG2 (Human liver hepatocellular carcinoma cell line)cells labeling Cathepsin D with purified ab75852 at 1/20 dilution (10µg/ml) (red).

Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A Goat anti rabbit IgG (Alexa Fluorr[®] 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)

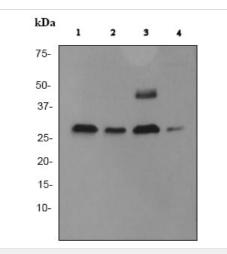
Lane 2 (+): ab75852 + SK-BR-3 whole cell lysate (10µg).

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

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution.

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 paraffinembedded 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.

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



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

Why choose a recombinant antibody? Research with Long-term and confidence scalable supply Consistent and Recombinant technology reproducible results Success from the Ethical standards first experiment compliant Confirmed Animal-free production specificity Anti-Cathepsin D antibody [EPR3057Y] (ab75852)

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

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