

Product datasheet

Anti-Cathepsin D antibody [EPR3056Y] ab75811

KO VALIDATED Recombinant RabMAB

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Overview

Product name	Anti-Cathepsin D antibody [EPR3056Y]
Description	Rabbit monoclonal [EPR3056Y] to Cathepsin D
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), ICC/IF, WB, IHC-P
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide within Human Cathepsin D aa 50-150 (N terminal). The exact sequence is proprietary.
Positive control	WB: A431, HepG2 and MCF7 whole cell lysate. MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate. IHC-P: Human thyroid cancer tissue. ICC/IF: MCF-7 cells. Flow Cyt (intra): MCF-7 cells.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAB[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAB[®] patents.</p> <p>Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.</p>

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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR3056Y
Isotype	IgG

Applications

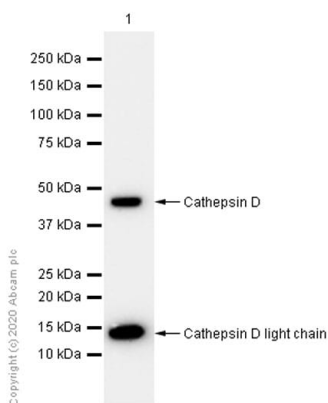
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab75811 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/20.
ICC/IF		1/50. For unpurified use at 1/100 - 1/250.
WB		1/1000. Detects a band of approximately 44 kDa (predicted molecular weight: 44 kDa). For unpurified use at 1/1000 - 1/2000.
IHC-P	★★★★★ (1)	1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See IHC antigen retrieval protocols . For unpurified use at 1/500.

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



Western blot - Anti-Cathepsin D antibody [EPR3056Y] (ab75811)

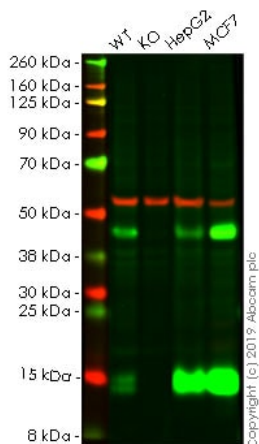
Anti-Cathepsin D antibody [EPR3056Y] (ab75811) at 1/1000 dilution + MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate at 15 µg

Secondary

Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/20000 dilution

Predicted band size: 44 kDa

Observed band size: 14,44 kDa



Western blot - Anti-Cathepsin D antibody [EPR3056Y] (ab75811)

All lanes : Anti-Cathepsin D antibody [EPR3056Y] (ab75811) at 1/1000 dilution (Unpurified)

Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 2 : CTSD knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 3 : Hep G2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

Lane 4 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 40 µg per lane.

Performed under reducing conditions.

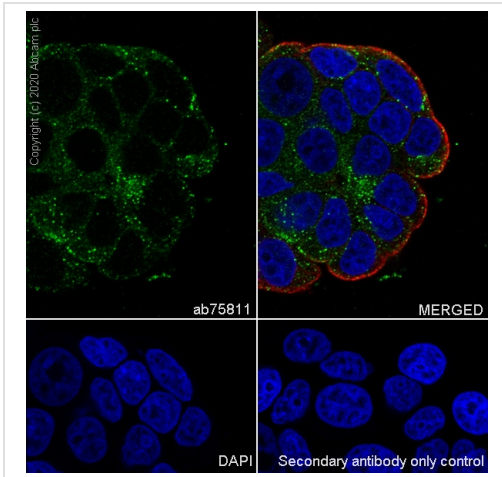
Predicted band size: 44 kDa

Observed band size: 44 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab75811 observed at 44 kDa. Red - loading control, **ab7291**, observed at 55 kDa.

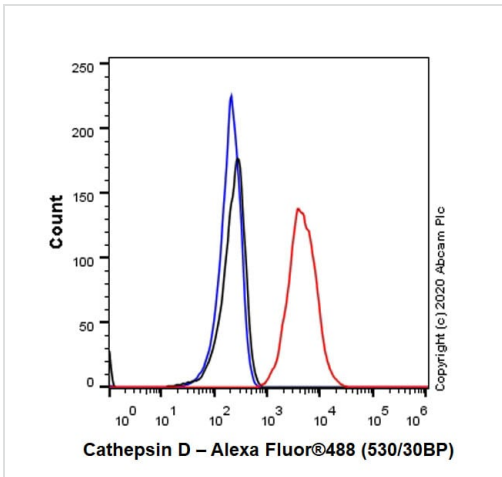
ab75811 was shown to specifically react with in wild-type A431

cells as signal was lost in CTSD knockout cells. Wild-type and CTSD knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% NF Milk. Ab75811 and **ab7291** (Mouse anti Tubulin loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



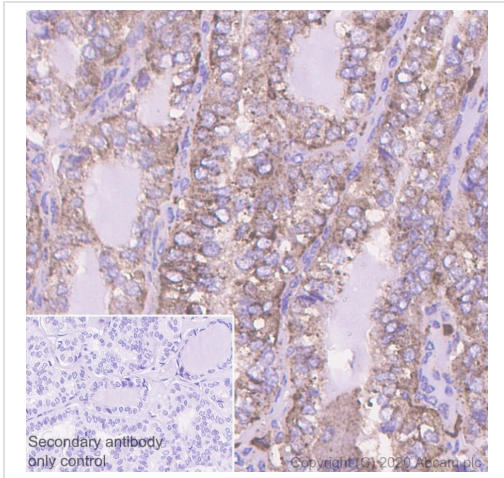
Immunocytochemistry/ Immunofluorescence - Anti-Cathepsin D antibody [EPR3056Y] (ab75811)

Immunocytochemistry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling Cathepsin D with purified ab75811 at 1/50 dilution (1.88 µg/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/mL). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/mL) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-Cathepsin D antibody [EPR3056Y] (ab75811)





Intracellular Flow Cytometry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling Cathepsin D with purified ab75811 at 1/20 dilution (10 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid cancer tissue sections labeling Cathepsin D with purified ab75811 at 1/100 dilution (0.94 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 1 (pH 6.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cathepsin D antibody [EPR3056Y] (ab75811)

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

Anti-Cathepsin D antibody [EPR3056Y] (ab75811)

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

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