abcam

Product datasheet

Anti-proCathepsin D antibody [EPR3054] ab134169

KO VALIDATED Recombinant RabMAb

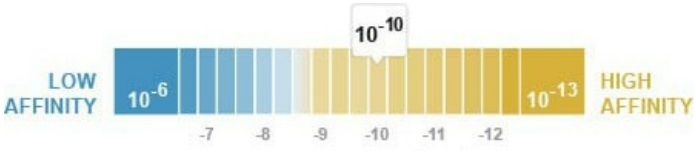
1 References 9 Images

Overview

Product name	Anti-proCathepsin D antibody [EPR3054]
Description	Rabbit monoclonal [EPR3054] to proCathepsin D
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human proCathepsin D aa 1-100. The exact sequence is proprietary.
Positive control	Human breast ductal infiltrating carcinoma tissue; A431, MCF7 and SKBR3 cell lysates.
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 **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Dissociation constant (K_D)	$K_D = 1.16 \times 10^{-10}$ M



Learn more about K_D

Storage buffer	pH: 7.2

	Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3054
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab134169 in the following tested applications.

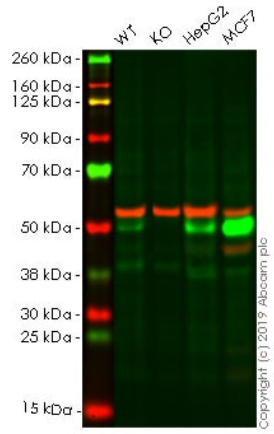
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		1/1000 - 1/10000. Predicted molecular weight: 44 kDa.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/100.

Target

Function	Acid protease active in intracellular protein breakdown. Involved in the pathogenesis of several diseases such as breast cancer and possibly Alzheimer disease.
Involvement in disease	Defects in CTSD are the cause of neuronal ceroid lipofuscinosis type 10 (CLN10) [MIM:610127]; also known as neuronal ceroid lipofuscinosis due to cathepsin D deficiency. A form of neuronal ceroid lipofuscinosis with onset at birth or early childhood. Neuronal ceroid lipofuscinoses are progressive neurodegenerative, lysosomal storage diseases characterized by intracellular accumulation of autofluorescent liposomal material, and clinically by seizures, dementia, visual loss, and/or cerebral atrophy.
Sequence similarities	Belongs to the peptidase A1 family.
Cellular localization	Lysosome. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

Images



Western blot - Anti-proCathepsin D antibody [EPR3054] (ab134169)

All lanes : Anti-proCathepsin D antibody [EPR3054] (ab134169) at 1/2000 dilution

Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 2 : Cathepsin D 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

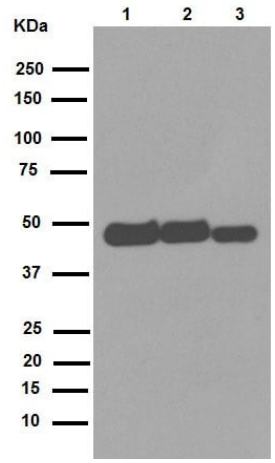
Lysates/proteins at 40 µg per lane.

Performed under reducing conditions.

Predicted band size: 44 kDa
Observed band size: 46 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab134169 observed at 46 kDa. Red - loading control, **ab7291** (mouse anti-tubulin), observed at 50 kDa.

ab134169 was shown to recognize in wild-type A431 cells as signal was lost at the expected MW in CTSD knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and CTSD knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% NF Milk. Ab134169 and **ab7291** (Mouse anti-tubulin loading control) were incubated overnight at 4°C at 1/2000 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.



Western blot - Anti-proCathepsin D antibody [EPR3054] (ab134169)

All lanes : Anti-proCathepsin D antibody [EPR3054] (ab134169) at 1/2000 dilution (purified)

Lane 1 : MCF-7 cell lysate
Lane 2 : A431 cell lysate
Lane 3 : SKBR-3 cell lysate

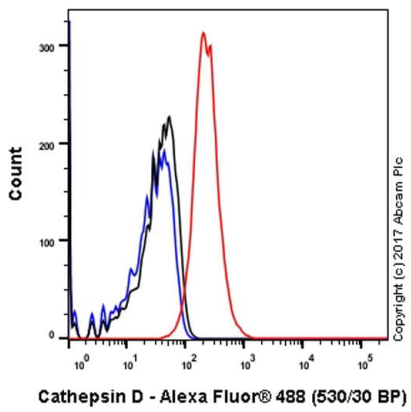
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 44 kDa
Observed band size: 44 kDa

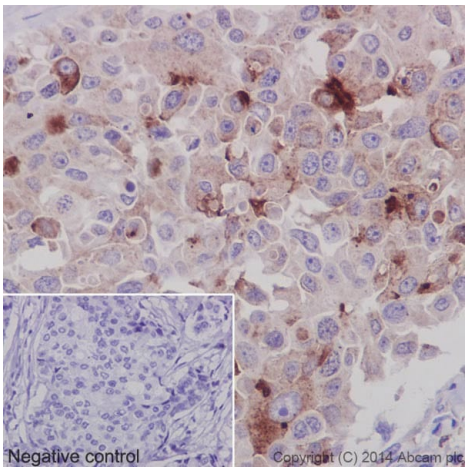
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



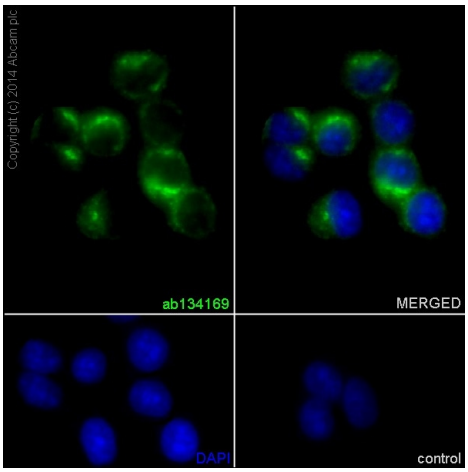
Flow Cytometry (Intracellular) - Anti-proCathepsin D antibody [EPR3054] (ab134169)

Intracellular Flow Cytometry analysis of A431 (human epidermoid carcinoma) cells labeling proCathepsin D with purified ab134169 at 1/100 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised 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.



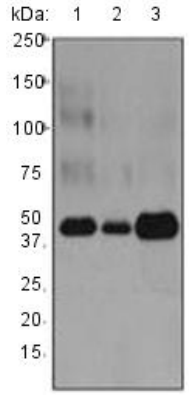
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-proCathepsin D antibody [EPR3054] (ab134169)

Immunohistochemical staining of paraffin embedded human breast carcinoma with purified ab134169 at a working dilution of 1 in 50. The secondary antibody used is a HRP polymer for rabbit IgG. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Immunocytochemistry/ Immunofluorescence - Anti-proCathepsin D antibody [EPR3054] (ab134169)

Immunofluorescence staining of MCF7 cells with purified ab134169 at a working dilution of 1 in 50, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti rabbit (**ab150077**), used at a dilution of 1 in 500. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative control is shown in bottom right hand panel - for the negative control, purified ab134169 was used at a dilution of 1/200 followed by an Alexa Fluor® 594 goat anti-mouse antibody at a dilution of 1/500.



Western blot - Anti-proCathepsin D antibody [EPR3054] (ab134169)

All lanes : Anti-proCathepsin D antibody [EPR3054] (ab134169) at 1/2000 dilution (unpurified)

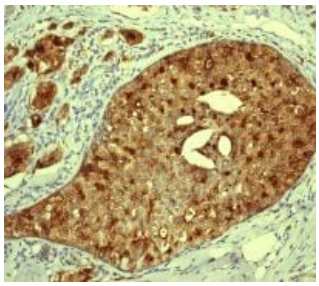
Lane 1 : MCF7 cell lysate
Lane 2 : A431 cell lysate
Lane 3 : SKBR3 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Standard HRP labelled goat anti-rabbit at 1/2000 dilution

Developed using the ECL technique.

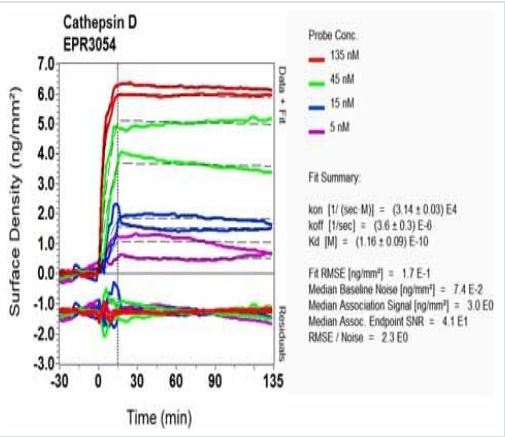
Predicted band size: 44 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-proCathepsin D antibody [EPR3054] (ab134169)

Immunohistochemical analysis of paraffin-embedded Human breast ductal infiltrating carcinoma tissue, staining proCathepsin D using unpurified ab134169 at a 1/250 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



OI-RD Scanning - Anti-proCathepsin D antibody [EPR3054] (ab134169)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

Anti-proCathepsin D antibody [EPR3054] (ab134169)

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