abcam

Product datasheet

Anti-Cathepsin D antibody [EPR3057Y] - Chicken IgY (Chimeric) ab302646

Recombinant

9 Images

Overview

Product name Anti-Cathepsin D antibody [EPR3057Y] - Chicken lgY (Chimeric)

Description Chicken monoclonal [EPR3057Y-CklgY] to Cathepsin D

Host species Chicken chimera

Tested applications Suitable for: WB, ICC/IF, Flow Cyt (Intra), IHC-P

Unsuitable for: IP

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Whole cell lysates: MCF7, SH-SY5Y, SK-BR-3, U937, A431, HepG2, RAW264.7, Neuro-2a;

Tissue lysates: Human heart, mouse brain, rat stomach. IHC-P: Human liver, breast carcinoma. ICC/IF: HepG2, Neuro-2a (mouse neuroblastoma neuroblast). Flow cyt. intr.: HepG2, Neuro-2a

(mouse neuroblastoma neuroblast).

General notes This chicken monoclonal chimeric antibody has been engineered from RabMab parent antibody

(<u>ab75852</u>). By necessity, some rabbit sequence is retained as part of the variable domain. When multiplexing with other rabbit-derived antibodies, using across absorbed Fc-reactive secondary

antibodies are recommended.

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.

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

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Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Thiophilic Chromatography

Clonality Monoclonal

Clone number EPR3057Y-CklgY

Isotype IgY

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab302646 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
WB		1/1000. Detects a band of approximately 14,28,46 kDa (predicted molecular weight: 44 kDa).
ICC/IF		1/50.
Flow Cyt (Intra)		1/1000.
IHC-P		1/10000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Application notes Is unsuitable for IP.

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

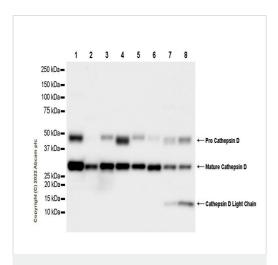
N- and O-glycosylated.

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-Cathespin D antibody

[EPR3057Y] - Chicken IgY (Chimeric) (ab302646)

All lanes : Anti-Cathepsin D antibody [EPR3057Y] - Chicken lgY (Chimeric) (ab302646) at 1/1000 dilution

Lane 1 : MCF7 (human breast adenocarcinoma epithelial cell), whole cell lysate

Lane 2: SH-SY5Y (human neuroblastoma epithelial cell), whole cell lysate

Lane 3 : SK-BR-3 (human breast adenocarcinoma epithelial cell), whole cell lysate

Lane 4: U937 (human histiocytic lymphoma monocyte), whole cell lysate

Lane 5: A431 (human epidermoid carcinoma epithelial cell), whole cell lysate

Lane 6: HepG2 (human hepatocellular carcinoma epithelial cell), whole cell lysate

Lane 7: RAW264.7 (mouse Abelson murine leukemia virusinduced tumor macrophage), whole cell lysate

Lane 8 : Neuro-2a (mouse neuroblastoma neuroblast), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

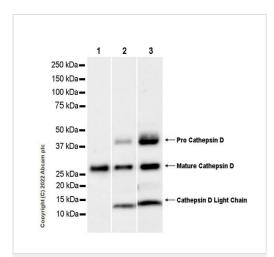
All lanes : Goat Anti-Chicken lgY H&L (HRP) (<u>ab6877</u>) at 1/20000 dilution

Predicted band size: 44 kDa

Observed band size: 14, 28, 46 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

Exposure time: 5.5 seconds.



Western blot - Anti-Cathespin D antibody
[EPR3057Y] - Chicken IgY (Chimeric) (ab302646)

All lanes : Anti-Cathepsin D antibody [EPR3057Y] - Chicken lgY (Chimeric) (ab302646) at 1/1000 dilution

Lane 1 : Human heart tissue lysate
Lane 2 : Mouse brain tissue lysate
Lane 3 : Rat stomach tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Chicken lgY H&L (HRP) (<u>ab6877</u>) at 1/20000 dilution

Predicted band size: 44 kDa

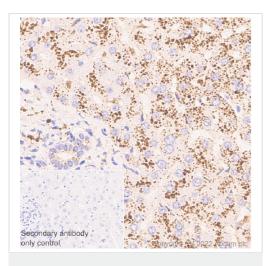
Observed band size: 14, 28, 46 kDa

Exposure time: 10 seconds

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling CTSD (cathepsin D) with AB302646 at 1/10000 dilution (0.08 µg/mL) followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Cytoplasmic with granular staining on human liver is observed. The section was incubated with ab302646 for 30 mins at room temperature, followed by Anti-Chicken lgY antibody (ab97136) for 8 mins during the LeicaDS9800 kit staining procedure. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

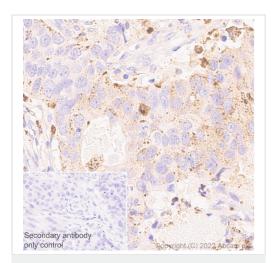
Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins was used.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cathespin D antibody

[EPR3057Y] – Chicken IgY (Chimeric) (ab302646)



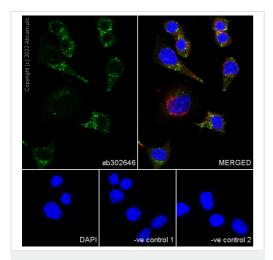
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cathespin D antibody

[EPR3057Y] - Chicken IgY (Chimeric) (ab302646)

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue labeling CTSD (cathepsin D) with AB302646 at 1/10000 dilution (0.08 µg/mL) followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Cytoplasmic with granular staining on human breast carcinoma is observed. The section was incubated with ab302646 for 30 mins at room temperature, followed by Anti-Chicken lgY antibody (ab97136) for 8 mins during the LeicaDS9800 kit staining procedure. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection).

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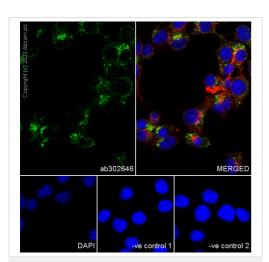


Immunocytochemistry/ Immunofluorescence - Anti-Cathespin D antibody [EPR3057Y] - Chicken IgY (Chimeric) (ab302646)

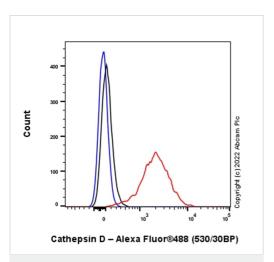
Immunofluorescent analysis of 100% methanol-fixed, 0.1% TritonX-100 permeabilized HepG2 (human hepatocellular carcinoma epithelial cell) cells labeling CTSD (cathepsin D) with AB302646 at 1/100 dilution (8.1 μ g/ml), followed by <u>ab150173</u> Goat Anti-Chicken lgY H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (2 μ g/mL) (Green). Confocal image showing cytoplasmic staining in HepG2 cell line. <u>ab179513</u> Anti-beta Tubulin rabbit monoclonal antibody was used to counterstain tubulin at 1/200 dilution (10 μ g/ml) (Red). The Nuclear counterstain was DAPI (Blue).

The negative controls are as follows:

-ve control 1: ab302646 was used as primary antibody at 1/50 dilution, followed by <u>ab150080</u> at 1/500 dilution (4 μ g/mL). -ve control 2: <u>ab179513</u> at 1/200 dilution (10 μ g/mL) was used as a primary antibody, followed by <u>ab150173</u> at 1/1000 (2 μ g/mL).



Immunocytochemistry/ Immunofluorescence - Anti-Cathespin D antibody [EPR3057Y] - Chicken IgY (Chimeric) (ab302646)



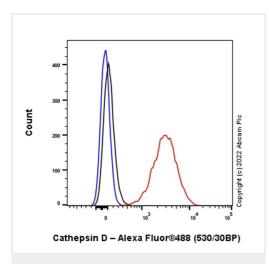
Flow Cytometry (Intracellular) - Anti-Cathespin D antibody [EPR3057Y] - Chicken IgY (Chimeric) (ab302646)

Immunofluorescent analysis of 100% methanol-fixed, 0.1% TritonX-100 permeabilized Neuro-2a (mouse neuroblastoma neuroblast) cells labeling CTSD (cathepsin D) with AB302646 at 1/100 dilution (8.1 μ g/ml), followed by <u>ab150173</u> Goat Anti-Chicken lgY H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (2 μ g/mL) (Green). Confocal image showing cytoplasmic staining in Neuro-2a (mouse neuroblastoma neuroblast) cell line. <u>ab179513</u> Anti-beta Tubulin rabbit monoclonal antibody was used to counterstain tubulin at 1/200 dilution (10 μ g/ml) (Red). The Nuclear counterstain was DAPI (Blue).

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Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized HepG2 (human hepatocellular carcinoma epithelial cell) cells labeling CTSD (cathepsin D) with AB302646 at 1/1000 dilution (0.1 μ g) (Red) compared with a Chicken lgY (Black) isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti-Chicken lgY H&L (Alexa Fluor® 488, **ab150173**) at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-Cathespin D antibody [EPR3057Y] - Chicken IgY (Chimeric) (ab302646)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized Neuro-2a (mouse neuroblastoma neuroblast) cells labeling CTSD (cathepsin D) with AB302646 at 1/1000 dilution (0.1 μ g) (Red) compared with a Chicken lgY (Black) isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti-Chicken lgY H&L (Alexa Fluor 488, **ab150173**) at 1/2000 dilution was used as the secondary antibody.



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