abcam

Product datasheet

Anti-Cathepsin D antibody ab72915



★★★★★ 1 Abreviews 6 References 画像数 4

製品の概要

製品名 Anti-Cathepsin D antibody

製品の詳細 Rabbit polyclonal to Cathepsin D

由来種 Rabbit

アプリケーション 適用あり: WB, ICC/IF, IHC-P

種交差性 交差種: Human

交差が予測される動物種: Dog, Pig, Orangutan 🔷

免疫原 Synthetic peptide corresponding to Human Cathepsin D aa 200-300 conjugated to keyhole limpet

haemocyanin.

(Peptide available as ab90605)

ポジティブ・コントロール WB: MCF7, A431 and HepG2 whole cell lysates. ICC/IF: methanol fixed HepG2 cells. IHC-P:

human adrenal gland tissue.

特記事項

The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -

80°C. Avoid freeze / thaw cycle.

バッファー pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our

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scientific support team who will be happy to help.

精製度 Immunogen affinity purified

ポリ/モノ ポリクローナル

アイソタイプ lgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab72915の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB	****(1)	Use a concentration of 1 µg/ml. Detects a band of approximately 45 kDa (predicted molecular weight: 45 kDa).
ICC/IF		Use a concentration of 5 µg/ml.
IHC-P		Use a concentration of 0.1 μ g/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

ターゲット情報

機能 Acid protease active in intracellular protein breakdown. Involved in the pathogenesis of several

diseases such as breast cancer and possibly Alzheimer disease.

組織特異性 Expressed in the aorta extrcellular space (at protein level).

関連疾患 Ceroid lipofuscinosis, neuronal, 10

配列類似性Belongs to the peptidase A1 family.
Contains 1 peptidase A1 domain.

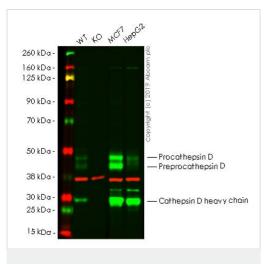
翻訳後修飾 N- and O-glycosylated.

細胞内局在 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).

画像



Western blot - Anti-Cathepsin D antibody (ab72915)

All lanes: Anti-Cathepsin D antibody (ab72915) at 1 µg/ml

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

Lane 4: Hep G2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 45 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab72915 observed at 28, 43, 46 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

ab72915 was shown to specifically react with CTSD 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% Milk. Ab72915 and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1 µg/ml 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-Cathepsin D antibody (ab72915)

All lanes: Anti-Cathepsin D antibody (ab72915) at 1 µg/ml

Lane 1 : MCF7 (Human breast adenocarcinoma cell line) Whole Cell Lysate

Lane 2 : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate

Lane 3: HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat polyclonal to Rabbit $\lg G$ - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

Predicted band size: 45 kDa

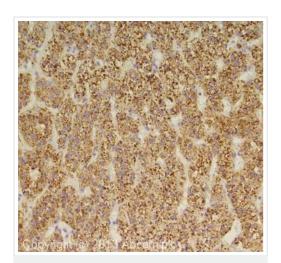
Observed band size: 29,45,48 kDa

Additional bands at: 150 kDa, 90 kDa. We are unsure as to the

identity of these extra bands.

Exposure time: 4 minutes

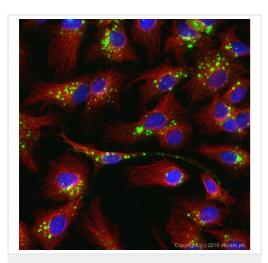
Cathepsin D has a predicted molecular weight of 45 kDa. The sequence contains a signal sequence and propeptide of 18 and 45 amino acids, respectively. This protein is further cleaved to produce a heavy and light chain with molecular weights of 27 kDa and 11 kDa, respectively (SwissProt). We hypothesize that the observed bands at 29 kDa represent the Cathepsin heavy chain, and the bands at 45 and 48 kDa represent the protein with and without the presence of the signal peptide.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cathepsin D antibody (ab72915)

IHC image of Cathepsin D staining in human adrenal gland formalin fixed paraffin embedded tissue section, performed on a Leica Bond system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab72915, 0.1µg/ml, 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.

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 (ab72915)

ICC/IF image of ab72915 stained HepG2 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Triton for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab72915 at 5 μ g/ml and ab7291 (Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control) at 1/1000 dilution overnight at +4°C. and ab7291 (Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control) at 1/1000 dilution overnight at +4°C. The secondary antibodies were ab150120 (pseudo-colored red) and ab150081 (colored green) used at 1 μ g/ml for 1 hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43 μ M for 1 hour at room temperature.

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