abcam

Product datasheet

Anti-CHMP2B antibody [EPR10807(B)] ab157208

KO VALIDATED Recombinant RabMAb

6 Images

Overview

Product name	Anti-CHMP2B antibody [EPR10807(B)]
Description	Rabbit monoclonal [EPR10807(B)] to CHMP2B
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IP, ICC/IF **Unsuitable for:** IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild-type A549 whole cell lysate, Hela, 293T, MCF7 whole cell lysates; Mouse brain lysate, Rat brain lysate, Rat heart lysate ICC/IF: HepG2 cells; Flow cyt: 293T cells; IP: 293T whole cell lysate.
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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20ºC.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR10807(B)

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab157208 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. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/10 - 1/100 dilution.
WB		1/1000. Predicted molecular weight: 24 kDa. For unpurified use at 1/10000 - 1/10000 dilution.
IP		1/20. For unpurified use at 1/10 - 1/100 dilution.
ICC/IF		1/250. For unpurified use at 1/250 - 1/500 dilution.

Application notes Is unsuitable for IHC-P.

Target

Function Probable core component of the endosomal sorting required for transport complex III (ESCRT-III) which is involved in multivesicular bodies (MVBs) formation and sorting of endosomal cargo proteins into MVBs. MVBs contain intraluminal vesicles (ILVs) that are generated by invagination and scission from the limiting membrane of the endosome and mostly are delivered to lysosomes enabling degradation of membrane proteins, such as stimulated growth factor receptors, lysosomal enzymes and lipids. The MVB pathway appears to require the sequential function of ESCRT-O, -I,-II and -III complexes. ESCRT-III proteins mostly dissociate from the invaginating membrane before the ILV is released. The ESCRT machinery also functions in topologically equivalent membrane fission events, such as the terminal stages of cytokinesis and the budding of enveloped viruses (HIV-1 and other lentiviruses). ESCRT-III proteins are believed to mediate the necessary vesicle extrusion and/or membrane fission activities, possibly in conjunction with the AAA ATPase VPS4.

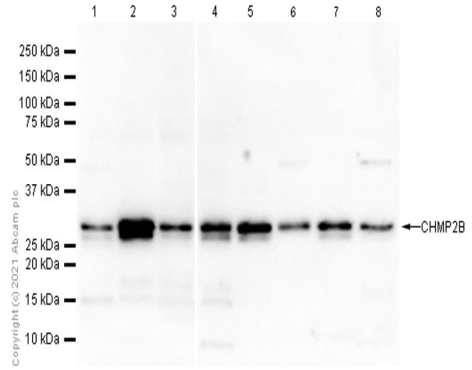
Tissue specificity Widely expressed. Expressed in brain, heart, skeletal muscle, spleen, kidney, liver, small intestine, pancreas, lung, placenta and leukocytes. In brain, it is expressed in cerebellum, cerebral cortex, medulla, spinal chord, occipital lobe, frontal lobe, temporal lobe and putamen.

Involvement in disease Defects in CHMP2B are the cause of frontotemporal dementia, chromosome 3-linked (FTD3) [MIM:600795]. FTD3 is characterized by an onset of dementia in the late 50's initially characterized by behavioral and personality changes including apathy, restlessness, disinhibition and hyperorality, progressing to stereotyped behaviors, non-fluent aphasia, mutism and dystonia, with a marked lack of insight. The brains of individuals with FTD3 have no distinctive neuropathological features. They show global cortical and central atrophy, but no beta-amyloid deposits.

Sequence similarities Belongs to the SNF7 family.

Domain	The acidic C-terminus and the basic N-termminus are thought to render the protein in a closed, soluble and inactive conformation through an autoinhibitory intramolecular interaction. The open and active conformation, which enables membrane binding and oligomerization, is achieved by interaction with other cellular binding partners, probably including other ESCRT components.
Cellular localization	Cytoplasm > cytosol. Late endosome membrane.

Images



Western blot - Anti-CHMP2B antibody [EPR10807(B)] (ab157208)

All lanes : Anti-CHMP2B antibody [EPR10807(B)] (ab157208) at 1/1000 dilution (Purified)

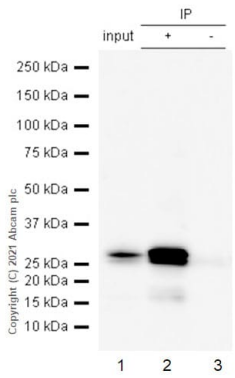
Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate
Lane 3 : A549 (Human lung carcinoma epithelial cell) whole cell lysate
Lane 4 : 293T (Human embryonic kidney epithelial cell) whole cell lysate
Lane 5 : Mouse brain lysate
Lane 6 : Mouse heart lysate
Lane 7 : Rat brain lysate
Lane 8 : Rat heart lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 24 kDa
Observed band size: 30 kDa

We are unsure about the nature of the 27kDa band. It may be isoform 2 of CHMP2B.

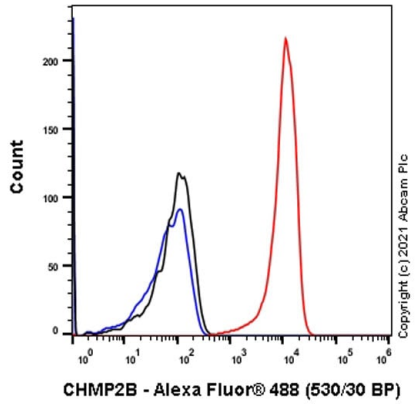


Immunoprecipitation - Anti-CHMP2B antibody [EPR10807(B)] (ab157208)

Purified ab157208 at 1:20 dilution (0.7µg) immunoprecipitating CHMP2B in 293T whole cell lysate.

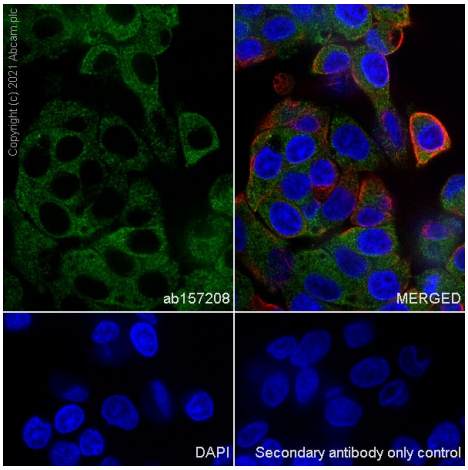
Lane 1 (input): 293T (Human embryonic kidney epithelial cell) whole cell lysate 10µg.
Lane 2 (+): ab157208 + 293T whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab157208 in 293T whole cell lysate.
VeriBlot for IP Detection Reagent (HRP)(**ab131366**) (1:1000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 30 kDa
We are unsure about the nature of the 27kDa band. It may be isoform 2 of CHMP2B.



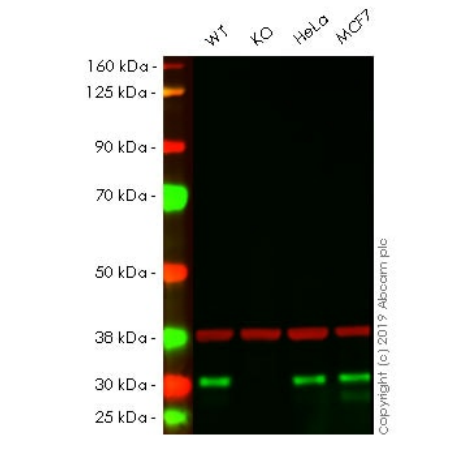
Flow Cytometry (Intracellular) - Anti-CHMP2B antibody [EPR10807(B)] (ab157208)

Flow Cytometry analysis of 293T (Human embryonic kidney epithelial cell) cells labelling CHMP2B with Purified ab157208 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). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-CHMP2B antibody [EPR10807(B)] (ab157208)

Immunocytochemistry analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling CHMP2B with Purified ab157208 at 1:250 dilution (0.6 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.



Western blot - Anti-CHMP2B antibody [EPR10807(B)] (ab157208)

All lanes : Anti-CHMP2B antibody [EPR10807(B)] (ab157208) at 1/1000 dilution

Lane 1 : Wild-type A549 whole cell lysate
Lane 2 : CHMP2B knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 4 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 24 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab157208 observed at 45 kDa. Red - loading control, **ab8245**, observed at 38 kDa.

ab157208 was shown to specifically react with CHMP2B in wild-

type A549 cells as signal was lost in CHMP2B knockout cells. Wild-type and CHMP2B knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% Milk. Ab157208 and **ab8245** (Mouse monoclonal [6C5] to GAPDH - Loading Control) were incubated overnight at 4°C at 1/1000 dilution and 1/1000 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.