abcam

Product datasheet

Anti-CLC3 antibody [N258/5] ab134285

6 References 4 Images

Overview

Product name	Anti-CLC3 antibody [N258/5]
Description	Mouse monoclonal [N258/5] to CLC3
Host species	Mouse
Specificity	Does not cross-react with CLCN4 or CLCN5 (based on KO validation results).
Tested applications	**Suitable for:** Flow Cyt, WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Rat, Human
Immunogen	Synthetic peptide corresponding to Rat CLC3 aa 98-115 (N terminal). Sequence: CKDRERHRRINSKKKESA Database link: **NP_445815.2** Run BLAST with Run BLAST with
Positive control	WB: Rat brain membrane lysate. IHC-P: FFPE human hippocampus tissue sections. ICC/IF: SK-N-BE cells.
General notes	The clone number has been updated from S258-5 to N258/5, both clone numbers name the same antibody clone. 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

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20ºC.
Storage buffer	Preservative: 0.09% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine)
Purity	Protein G purified

Clonality	Monoclonal
Clone number	N258/5
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab134285 in the following tested applications.

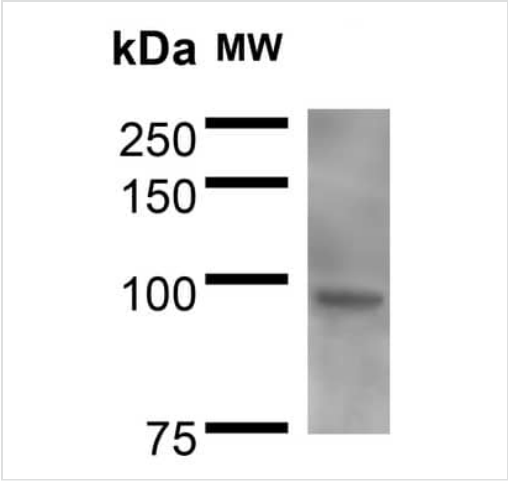
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt		Use 1µg for 10^6 cells. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
WB		1/1000. Predicted molecular weight: 91 kDa. 1µg/ml of ab134285 is sufficient for detection of CLC3 in 20µg of Rat brain membrane lysate.
IHC-P		1/1000.
ICC/IF		1/100.

Target

Function	Mediates the exchange of chloride ions against protons. Functions as antiporter and contributes to the acidification of the endosome and synaptic vesicle lumen, and may thereby affect vesicle trafficking and exocytosis. May play an important role in neuronal cell function through regulation of membrane excitability by protein kinase C. It could help neuronal cells to establish short-term memory.
Tissue specificity	Expressed primarily in tissues derived from neuroectoderm. Within the brain, its expression is particularly evident in the hippocampus, olfactory cortex, and olfactory bulb. Highly expressed in aortic and coronary vascular smooth muscle cells, and aortic endothelial cells. Also expressed in tracheal and alveolar epithelial cells, and intima and media of the pulmonary vessels. Expressed in bronchus and colon (at protein level).
Sequence similarities	Belongs to the chloride channel (TC 2.A.49) family. ClC-3/CLCN3 subfamily. Contains 2 CBS domains.
Domain	Isoform 2 contains a C-terminal PDZ-binding motif mediating the interaction with GOPC.
Post-translational modifications	N-glycosylated.
Cellular localization	Membrane. Early endosome membrane. Late endosome membrane. Cytoplasmic vesicle > secretory vesicle membrane. Isoform 1 is localized mainly in early and late endosomes and Membrane. Early endosome membrane. Late endosome membrane. Golgi apparatus membrane. Isoform 2 partially colocalized with isoform 1 but is mainly enriched in the Golgi.

Images



Western blot - Anti-CLC3 antibody [N258/5] (ab134285)

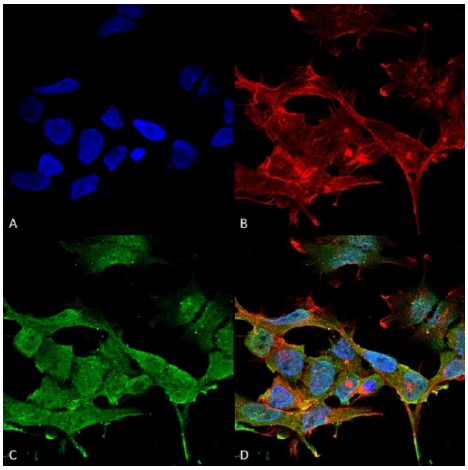
Anti-CLC3 antibody [N258/5] (ab134285) at 1/200 dilution + Rat brain membrane lysate at 15 µg

Secondary

Goat Anti-Mouse IgG: HRP at 1/1000 dilution

Developed using the ECL technique.

Predicted band size: 91 kDa

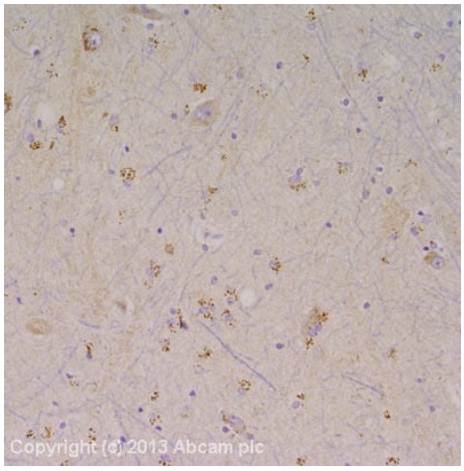


Immunocytochemistry/ Immunofluorescence - Anti-CLC3 antibody [N258/5] (ab134285)

4% formaldehyde-fixed SK-N-BE cells stained for CLC3 (green) using ab134285 at 1/100 dilution in ICC/IF.

Secondary Antibody: Goat Anti-Mouse ATTO 488 at 1/100 for 60 minutes at room temperature. Counterstain: Phalloidin Texas Red F-Actin stain; DAPI (blue) nuclear stain at 1/1000, 1/5000 for 60 minutes room temperature, 5 minutes room temperature.

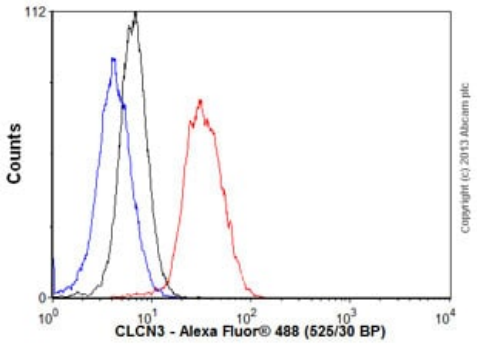
(A) DAPI (blue) nuclear stain. (B) Phalloidin Texas Red F-Actin stain. (C) ab134285. (D) Composite.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CLC3 antibody [N258/5] (ab134285)

IHC image of CLC3 staining in human hippocampus 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 ab134285, 10µg/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.



Flow Cytometry - Anti-CLC3 antibody [N258/5] (ab134285)

Overlay histogram showing SH-SY5Y cells stained with ab134285 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab134285, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse IgG (H&L) (**ab150113**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

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