abcam

Product datasheet

Anti-SNAP25 antibody [4A3] ab66066

8 References 4 Images

Overview

Product name	Anti-SNAP25 antibody [4A3]
Description	Mouse monoclonal [4A3] to SNAP25
Host species	Mouse
Tested applications	**Suitable for:** WB, ICC/IF, Flow Cyt
Species reactivity	**Reacts with:** Mouse, Human, Zebrafish **Predicted to work with:** Rat, Chicken, Dog, Xenopus laevis, Cynomolgus monkey, Opossum, Orangutan
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: SNAP25 transfected 293T cell lysate; Zebrafish brain homogenate; SH-SY5Y; Mouse brain homogenate. IHC-P: SKNSH cells. Flow cyt: SH-SY5Y cells.
General notes	This product was changed from ascites to tissue culture supernatant on 4th April 2019. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team. 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. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer	pH: 7.40 Constituent: 100% PBS
Purity	Protein A purified
Clonality	Monoclonal

Clone number	4A3
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab66066 in the following tested applications.

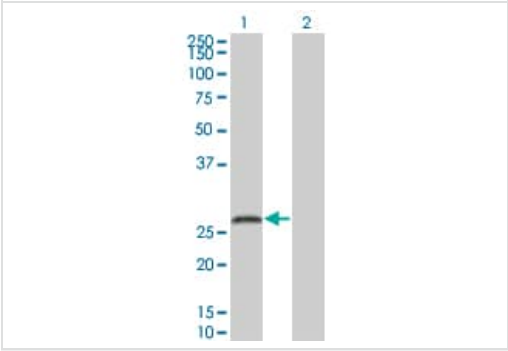
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 27 kDa (predicted molecular weight: 23 kDa).
ICC/IF		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Target

Function	t-SNARE involved in the molecular regulation of neurotransmitter release. May play an important role in the synaptic function of specific neuronal systems. Associates with proteins involved in vesicle docking and membrane fusion. Regulates plasma membrane recycling through its interaction with CENPF.
Tissue specificity	Neurons of the neocortex, hippocampus, piriform cortex, anterior thalamic nuclei, pontine nuclei, and granule cells of the cerebellum.
Sequence similarities	Belongs to the SNAP-25 family. Contains 2 t-SNARE coiled-coil homology domains.
Post-translational modifications	Palmitoylated. Cys-85 appears to be the main site, and palmitoylation is required for membrane association.
Cellular localization	Cytoplasm > perinuclear region. Cell membrane. Cell junction > synapse > synaptosome. Membrane association requires palmitoylation. Expressed throughout cytoplasm, concentrating at the perinuclear region.

Images



Western blot - Anti-SNAP25 antibody [4A3] (ab66066)

All lanes : Anti-SNAP25 antibody [4A3] (ab66066) at 1 µg/ml

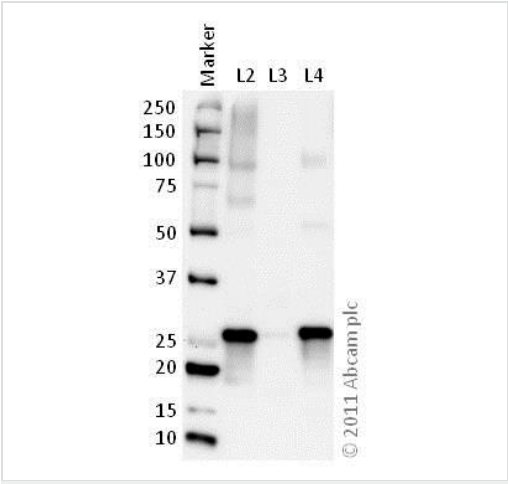
Lane 1 : SNAP25 transfected 293T cell lysate
Lane 2 : Non-transfected 293T cell lysate

Lysates/proteins at 50 µg per lane.

Secondary
All lanes : Goat Anti-Mouse IgG (H&L)-HRP at 1/2500 dilution

Predicted band size: 23 kDa
Observed band size: 27 kDa

This image was generated using the ascites version of the product.



Western blot - Anti-SNAP25 antibody [4A3] (ab66066)

All lanes : Anti-SNAP25 antibody [4A3] (ab66066) at 1 µg/ml

Lane 1 : Marker
Lane 2 : Zebrafish brain homogenate at 20 µg
Lane 3 : SH-SY5Y (Human neuroblastoma cell line) whole cell lysate at 20 µg
Lane 4 : Mouse brain homogenate at 20 µg

Secondary
All lanes : Goat polyclonal to Mouse IgG – H&L – Pre-Adsorbed (HRP) at 1/6000 dilution

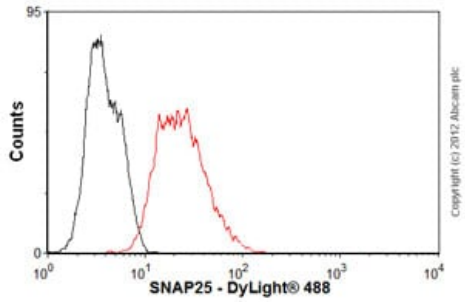
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 23 kDa
Observed band size: 26 kDa

Exposure time: 2 minutes

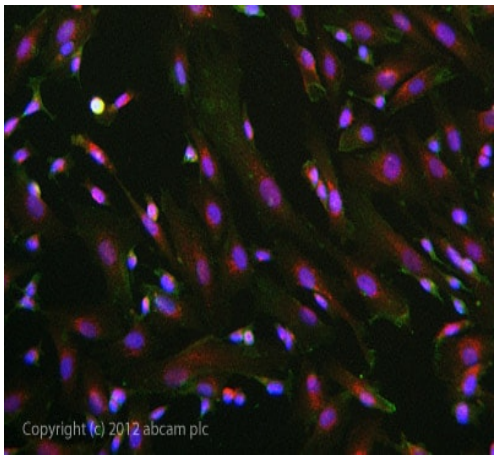
This image was generated using the ascites version of the product.



Flow Cytometry - Anti-SNAP25 antibody [4A3] (ab66066)

Overlay histogram showing SH-SY5Y cells stained with ab66066 (red line). The cells were fixed with 4% paraformaldehyde (10 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 (ab66066, 1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in SH-SY5Y cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

This image was generated using the ascites version of the product.



Immunocytochemistry/ Immunofluorescence - Anti-SNAP25 antibody [4A3] (ab66066)

ab66066 stained SKNSH cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab66066 at 5µg/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- mouse (**ab96879**) IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

This image was generated using the ascites version of the product.

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

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- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors