abcam

Product datasheet

Anti-SNX27 antibody [1C6] ab77799

10 Abreviews 38 References 4 Images

Overview

Product name	Anti-SNX27 antibody [1C6]
Description	Mouse monoclonal [1C6] to SNX27
Host species	Mouse
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IP, ICC/IF
Species reactivity	**Reacts with:** Human **Predicted to work with:** Monkey **Does not react with:** Mouse
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Epitope	N-terminal amino acids 1-267.
Positive control	This antibody gave a positive signal in the following whole cell lysates: HeLa; A431; HEK293; A549; MDA-MB-231. ICC/IF: Jurkat cells
General notes	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Purity	Protein G purified
Clonality	Monoclonal

Clone number	1C6
Myeloma	Sp2/0
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab77799 in the following tested applications.

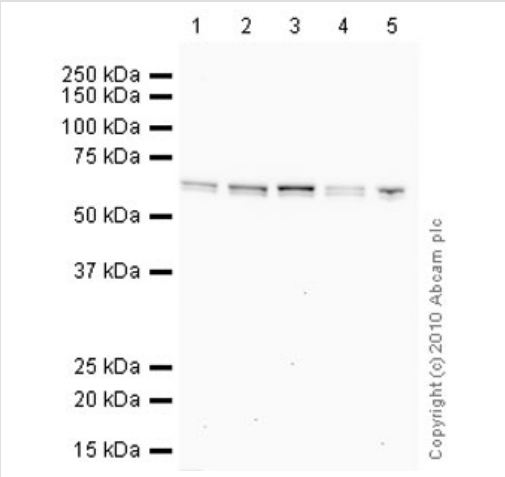
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use 0.1-1µg for 10^6 cells. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
WB	★★★★☆ **(6)**	Use a concentration of 10 µg/ml. Detects a band of approximately 61 kDa (predicted molecular weight: 61 kDa). Abcam recommends using 3% milk as the blocking agent.
IP		Use at an assay dependent concentration.
ICC/IF	★★★★☆ **(4)**	Use a concentration of 5 µg/ml.

Target

Function	Involved in endocytic trafficking (By similarity). In T lymphocytes, participates in endocytic recycling pathway. Recruits PSCDBP and HT4R to early endosomes.
Tissue specificity	Expressed in cells of hematopoietic origin (at protein level).
Sequence similarities	Belongs to the sorting nexin family. Contains 1 PDZ (DHR) domain. Contains 1 PX (phox homology) domain. Contains 1 Ras-associating domain.
Domain	The PDZ domain mediates the interaction with DGKZ, PSCDBP and HT4R and is responsible for vesicular localization.
Cellular localization	Cytoplasm > cytosol. Early endosome. In T-lymphocytes, recruited from the cytosol to sorting endosomes by phosphoinositide-3-kinase products.

Images



Western blot - Anti-SNX27 antibody [1C6] (ab77799)

All lanes : Anti-SNX27 antibody [1C6] (ab77799) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 3 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate
Lane 4 : A549 (Human lung adenocarcinoma epithelial cell line) Whole Cell Lysate
Lane 5 : MDA-MB-231 (Human breast adenocarcinoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

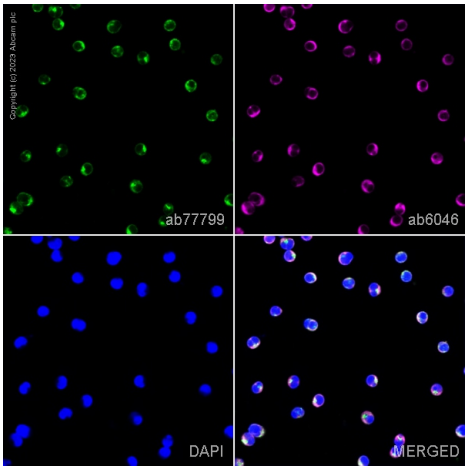
Performed under reducing conditions.

Predicted band size: 61 kDa
Observed band size: 61 kDa

Exposure time: 15 minutes

Blocking: 5% Milk in TBS-T.

Two bands are observed very close together, at around 60-kDa. There are four reported isoforms of the SNX27 protein (Swissprot). We hypothesise that this antibody detects both isoform 1 and isoform 2, which are 61-kDa and 60-kDa respectively. As the immunogen is derived from the N-terminal region of SNX27, the antibody is not expected to react with isoforms 3 or 4.

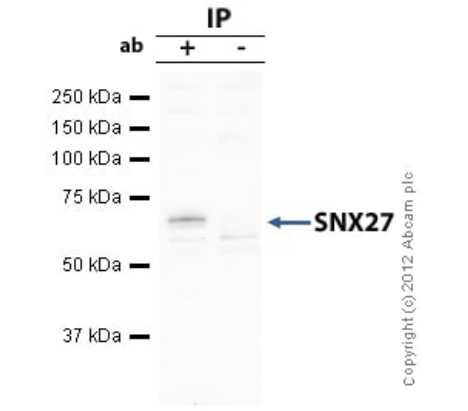


Immunocytochemistry/ Immunofluorescence - Anti-SNX27 antibody [1C6] (ab77799)

ab77799 staining SNX27 in Jurkat cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab77799 at 5µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour magenta). Nuclear DNA was labelled with DAPI (shown in blue). Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.

Flow Cytometry (Intracellular) - Anti-SNX27 antibody [1C6] (ab77799)

Overlay histogram showing Jurkat cells stained with ab77799 (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 (ab77799, 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 Jurkat cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunoprecipitation - Anti-SNX27 antibody [1C6] (ab77799)

SNX27 was immunoprecipitated using 0.5mg Hek293 whole cell extract, 5µg of Mouse monoclonal to SNX27 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, Hek293 whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70ºC; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab77799.
Secondary: Goat polyclonal to mouse IgG light chain specific (HRP) at 1/5000 dilution.

Band: 61kDa: SNX27.

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