abcam

Product datasheet

Anti-VPS35 antibody ab10099

KO VALIDATED

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Overview

Product name	Anti-VPS35 antibody
Description	Goat polyclonal to VPS35
Host species	Goat
Tested applications	**Suitable for:** IHC-P, WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat, Cow
Immunogen	Synthetic peptide corresponding to Human VPS35 aa 783-796 (C terminal). Sequence: C-SPESEGPIYEGLIL (Peptide available as **ab23181**) Run BLAST with Run BLAST with
Positive control	WB: Human and Mouse Brain lysate. HepG2 and HEK-293 whole cell lysates. IHC-P: Human testis tissue. ICC/IF: U2OS, HeLa, HEK293 cells. IHC-P: Human prostate tissue.
General notes	The maintenance of organelles in eukaryotic cells depends on sorting proteins, which ensure the proper delivery of organelle-specific proteins. In S. cerevisiae, VPS35 has a direct role in the retrieval of vacuolar cargo proteins, suggesting that human VPS35 may have an analogous function in the maintenance of lysosomes. 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: 7.30 Preservative: 0.02% Sodium azide Constituents: 99% Tris buffered saline, 0.5% BSA
Purity	Immunogen affinity purified
Purification notes	Purified from goat serum by ammonium sulphate precipitation followed by antigen affinity chromatography using the immunizing peptide.
Primary antibody notes	The maintenance of organelles in eukaryotic cells depends on sorting proteins, which ensure the proper delivery of organelle-specific proteins. In S. cerevisiae, VPS35 has a direct role in the retrieval of vacuolar cargo proteins, suggesting that human VPS35 may have an analogous function in the maintenance of lysosomes.
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab10099 in the following tested applications.

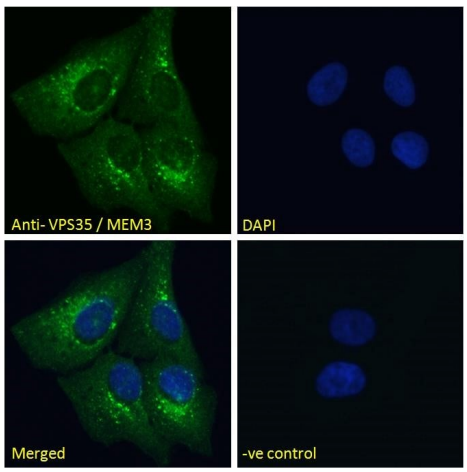
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		Use a concentration of 5 - 8 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB	★★★★☆ (2)	Use a concentration of 0.03 - 3 µg/ml. Detects a band of approximately 85 kDa (predicted molecular weight: 91 kDa). 1 hour primary incubation is recommended for this product.
ICC/IF	★★★★★ (1)	Use a concentration of 10 µg/ml.

Target

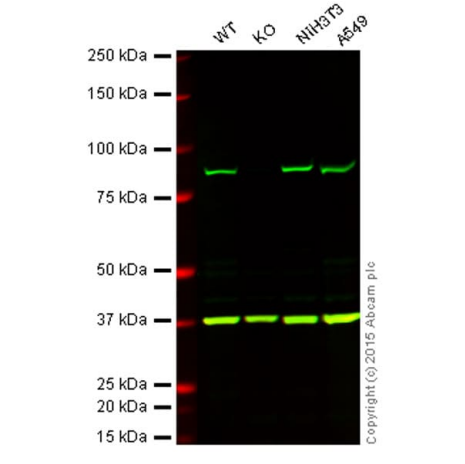
Function	Essential component of the retromer complex, a complex required to retrieve lysosomal enzyme receptors (IGF2R and M6PR) from endosomes to the trans-Golgi network. Also required to regulate transcytosis of the polymeric immunoglobulin receptor (pIgR-pIgA).
Tissue specificity	Ubiquitous. Highly expressed in heart, brain, placenta, skeletal muscle, spleen, thymus, testis, ovary, small intestine, kidney and colon.
Sequence similarities	Belongs to the VPS35 family.
Cellular localization	Cytoplasm. Membrane.

Images



Immunocytochemistry/ Immunofluorescence - Anti-VPS35 antibody (ab10099)

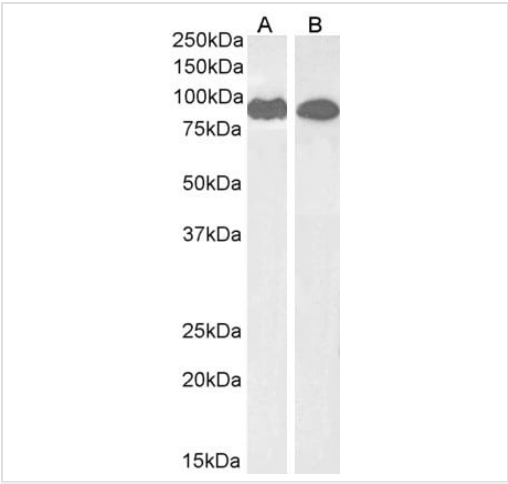
Immunocytochemistry/Immunofluorescence analysis of paraformaldehyde fixed U2OS cells, permeabilized with 0.15% Triton. Primary incubation 1hr (10µg/mL) followed by Alexa Fluor 488 secondary antibody (2µg/mL), showing cytoplasmic/vesicle staining. The nuclear stain is DAPI (blue).
Negative control: Unimmunized goat IgG (10µg/mL) followed by Alexa Fluor 488 secondary antibody (2µg/mL).



Western blot - Anti-VPS35 antibody (ab10099)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: VPS35 knockout HAP1 cell lysate (20 µg)
Lane 3: NIH3T3 cell lysate (20 µg)
Lane 4: A549 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab10099 observed at 85 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

ab10099 was shown to specifically react with VPS35 when VPS35 knockout samples were used. Wild-type and VPS35 knockout samples were subjected to SDS-PAGE. ab10099 and **ab181602** (loading control to GAPDH) were diluted to 1 µg/ml and 1/1000 respectively and incubated overnight at 4°C. Blots were developed with IRDye® 800CW Donkey anti-Goat IgG (H + L) and Donkey Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216779**) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.



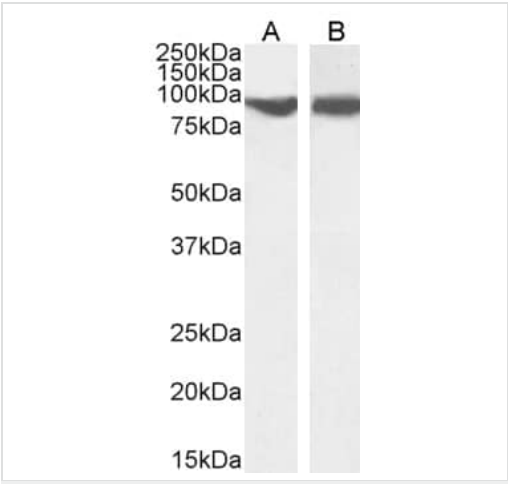
Western blot - Anti-VPS35 antibody (ab10099)

All lanes : Anti-VPS35 antibody (ab10099) at 1 µg/ml

Lane 1 : Human Cerebellum
Lane 2 : Mouse brain

Lysates/proteins at 35 µg per lane.

Predicted band size: 91 kDa



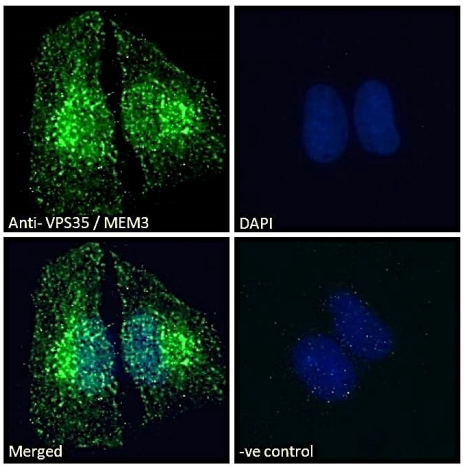
Western blot - Anti-VPS35 antibody (ab10099)

All lanes :

Lane 1 : HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate (35µg protein in RIPA buffer).
Lane 2 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
(35µg protein in RIPA buffer).

Predicted band size: 91 kDa

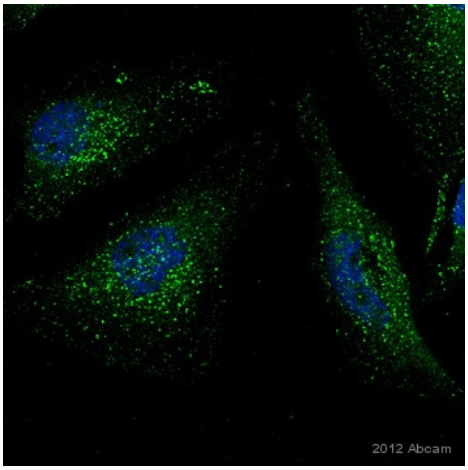
Detected by chemiluminescence.



Immunocytochemistry/ Immunofluorescence - Anti-VPS35 antibody (ab10099)

Immunofluorescence analysis of paraformaldehyde fixed HEK293 cells, permeabilized with 0.15% Triton. Primary incubation 1hr (10 ug/ml) followed by Alexa Fluor 488 secondary antibody (2 ug/ml), showing cytoplasmic/vesicle staining. The nuclear stain is DAPI (blue).

Negative control: Unimmunized goat IgG (10 ug/ml) followed by Alexa Fluor 488 secondary antibody (2 ug/ml).

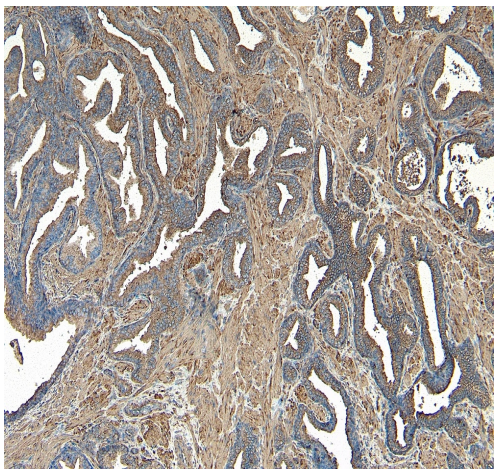


Immunocytochemistry/ Immunofluorescence - Anti-VPS35 antibody (ab10099)

This image is courtesy of an anonymous Abreview

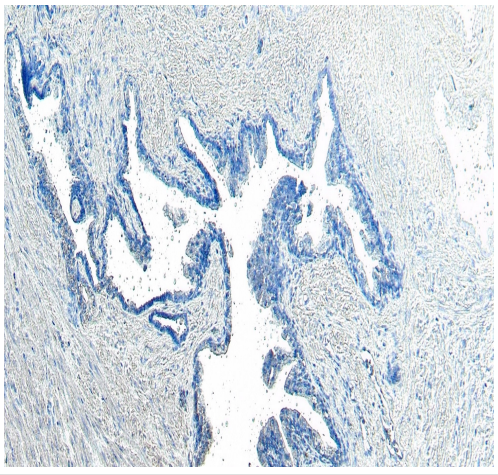
Immunofluorescence analysis of HeLa cells, staining VPS35 with ab10099.

Cells were fixed with paraformaldehyde, permeabilized with 0.1% Triton X-100 and blocked with 10% serum for 1 hour at 22°C. Samples were incubated with primary antibody (1/150 in 10% Serum in PBS) for 16 hours at 4°C. An AlexaFluor®488-conjugated donkey anti-goat polyclonal IgG (1/300) was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VPS35 antibody (ab10099)

Immunohistochemical analysis of paraffin embedded human prostate tissue labeling VPS35 with ab10099 at 8 ug/ml.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VPS35 antibody (ab10099)

Negative Control showing staining of paraffin embedded Human Prostate, with no primary antibody.

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