abcam

Product datasheet

Anti-VPS35 antibody ab118838

KO VALIDATED

4 Images

Overview

Product name	Anti-VPS35 antibody
Description	Rabbit polyclonal to VPS35
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, IHC-P
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat, Rabbit, Horse, Cow, Dog, Pig, Chimpanzee, Macaque monkey, Orangutan
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	This antibody gave a positive signal in the following whole cell lysates: HepG2; MOLT4; Raji. This antibody gave a positive result in IHC in the following FFPE tissue: Human normal heart muscle. This antibody gave a positive result when used in the following formaldehyde fixed cell lines: HepG2.
General notes	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 +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab118838 in the following tested applications.

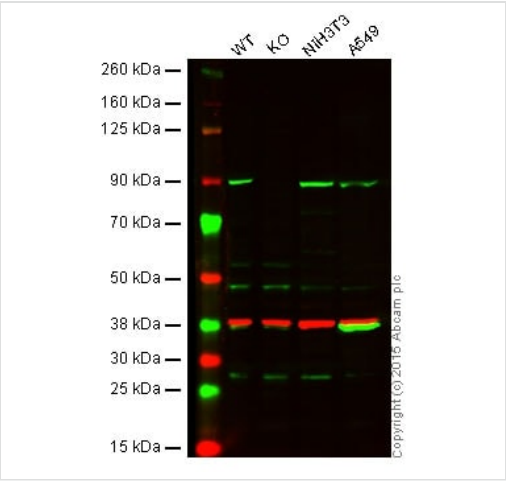
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 91 kDa (predicted molecular weight: 91 kDa).
ICC/IF		Use a concentration of 1 µg/ml.
IHC-P		Use a concentration of 5 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.

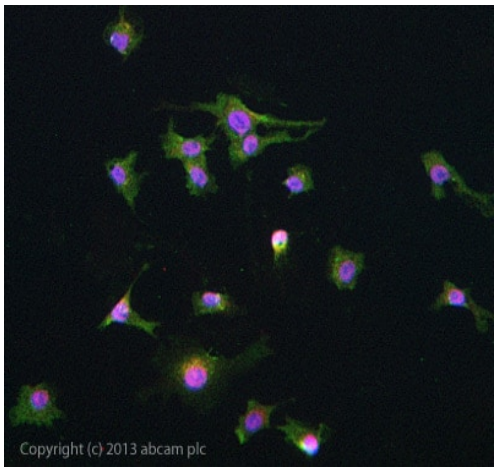
Images



Western blot - Anti-VPS35 antibody (ab118838)

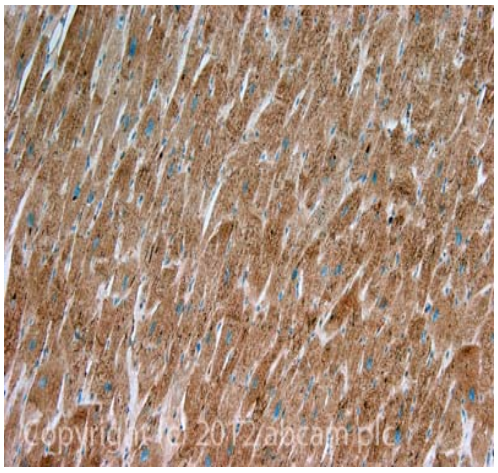
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: VPS35 knockout HAP1 cell lysate (20 µg)
Lane 3: NIH/3T3 cell lysate (20 µg)
Lane 4: A549 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab118838 observed at 90 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab118838 was shown to recognize VPS35 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when VPS35 knockout samples were examined. Wild-type and VPS35 knockout samples were subjected to SDS-PAGE. ab118838 and ab8245 (loading control to GAPDH) were diluted 1µg/mL and 1/2000 and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed ab216776 secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-VPS35 antibody (ab118838)

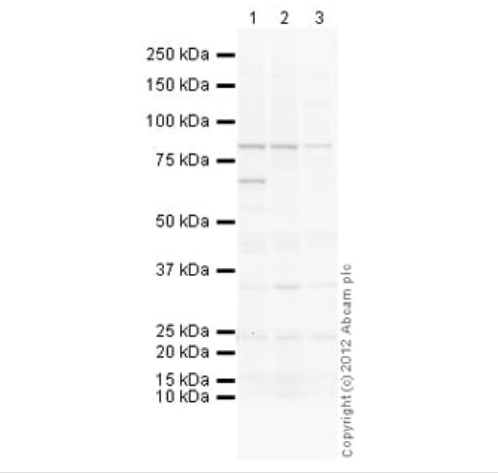
ICC/IF image of ab118838 stained HepG2 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab118838 at 1µg/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- rabbit (ab96899) IgG (H+L) used at a 1/250 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VPS35 antibody (ab118838)

IHC image of VPS35 staining in Human normal heart muscle formalin fixed paraffin embedded tissue section, performed on a Leica BondTM 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 ab118838, 5µg/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.



Western blot - Anti-VPS35 antibody (ab118838)

All lanes : Anti-VPS35 antibody (ab118838) at 1 µg/ml

Lane 1 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate
Lane 2 : MOLT4 (Human acute lymphoblastic leukemia cell line) Whole Cell Lysate
Lane 3 : Raji (Human Burkitt's lymphoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 91 kDa

Observed band size: 85 kDa

Additional bands at: 24 kDa, 35 kDa, 71 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 2 minutes

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