abcam

ab157220

Anti-VPS35 antibody [EPR11501(B)]

Anti-VPS35 antibody [EPR11501(B)] (ab157220) is a rabbit monoclonal antibody detecting VPS35 in **Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF**. Suitable for **Human, Mouse**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 20 publications

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 50% Tissue culture supernatant, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR11501(B)
Purification technique	Affinity purification Protein A
Concentration	0.5 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/50 - 1/100
Notes	Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IP

Not recommended

Species	Mouse, Human, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000 - 1/50000
Notes	-

Species	Mouse
Dilution info	1/1000 - 1/50000
Notes	-

Species	Rat
Dilution info	1/1000 - 1/50000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	0.2 µg/mL
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/10 - 1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Rat

Dilution info	-
Notes	-

Target data

See full target information VPS35

Function	Acts as a component of the retromer cargo-selective complex (CSC). The CSC is believed to be the core functional component of retromer or respective retromer complex variants acting to prevent missorting of selected transmembrane cargo proteins into the lysosomal degradation pathway. The recruitment of the CSC to the endosomal membrane involves RAB7A and SNX3. The CSC seems to associate with the cytoplasmic domain of cargo proteins predominantly via VPS35; however, these interactions seem to be of low affinity and retromer SNX proteins may also contribute to cargo selectivity thus questioning the classical function of the CSC. The SNX-BAR retromer mediates retrograde transport of cargo proteins from endosomes to the trans-Golgi network (TGN) and is involved in endosome-to-plasma membrane transport for cargo protein recycling. The SNX3-retromer mediates the retrograde endosome-to-TGN transport of WLS distinct from the SNX-BAR retromer pathway (PubMed:30213940). The SNX27-retromer is believed to be involved in endosome-to-plasma membrane trafficking and recycling of a broad spectrum of cargo proteins. The CSC seems to act as recruitment hub for other proteins, such as the WASH complex and TBC1D5 (Probable). Required for retrograde transport of lysosomal enzyme receptor IGF2R and SLC11A2. Required to regulate transcytosis of the polymeric immunoglobulin receptor (pIgR-pIgA) (PubMed:15078903, PubMed:15247922, PubMed:20164305). Required for endosomal localization of WASHC2C (PubMed:22070227, PubMed:28892079). Mediates the association of the CSC with the WASH complex via WASHC2 (PubMed:22070227, PubMed:24819384, PubMed:24980502). Required for the endosomal localization of TBC1D5 (PubMed:20923837). (Microbial infection) The heterotrimeric retromer cargo-selective complex (CSC) mediates the exit of human papillomavirus from the early endosome and the delivery to the Golgi apparatus.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C

Notes

What is this antibody validated in?
Anti-VPS35 antibody [EPR11501(B)] (ab157220) is a rabbit recombinant monoclonal antibody and is validated for use

in Western Blot (WB), Flow Cytometry (Intra), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human, Mouse samples.

What is the molecular weight of VPS35?
Anti-VPS35 [EPR11501(B)] (ab157220) specifically detects a band for VPS35 (UniProt: Q96QK1) at a molecular weight of 92kDa.

Trusted by the scientific community
Anti-VPS35 [EPR11501(B)] (ab157220) was first used in a scientific publication in 2013 and has been cited over 20 times in peer-reviewed journals.

Specificity confirmed
The specificity of Anti-VPS35 antibody [EPR11501(B)] (ab157220) has been confirmed by Western blot testing in VPS35 Knockout HAP1 cells.

Other related products
We have a range of other formats of antibody clone [EPR11501(B)] also available for your convenience: ab157220, HRP - ab215340, Carrier free - ab240141, APC - ab310829, PE - ab310905, Alexa Fluor® 594 - ab311695, Alexa Fluor® 568 - ab312970, Alexa Fluor® 555 - ab313179, Alexa Fluor® 750 - ab321149

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

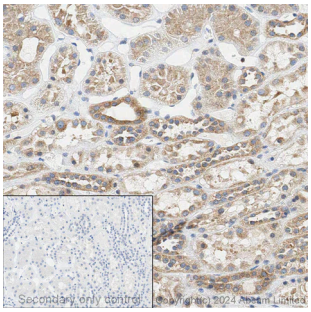
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

13 product images

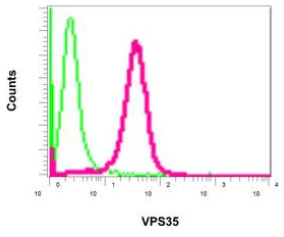


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VPS35 antibody [EPR11501(B)] (ab157220)

Immunohistochemical analysis of formalin fixed paraffin embedded human kidney labelling VPS35 with ab157220 at a concentration of 0.01µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with a OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was performed with DISCOVERY cell conditioning solution (CC1) 100°C, pH8.5 for 32mins.

ab157220 Anti-VPS35 antibody [EPR11501(B)] was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

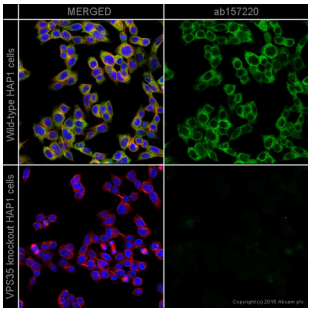
Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual).



Flow Cytometry (Intracellular) - Anti-VPS35 antibody [EPR11501(B)] (ab157220)

VPS35 Flow Cytometry (Intracellular) staining using rabbit Anti-VPS35 antibody

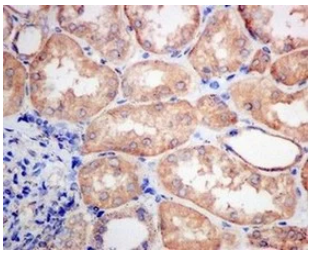
Intracellular flow cytometric analysis of permeabilized MOLT4 cells labeling VPS35 with ab157220 at 1/10 dilution (red) or a rabbit IgG (negative) (green).



Immunocytochemistry/ Immunofluorescence - Anti-VPS35 antibody [EPR11501(B)] (ab157220)

VPS35 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-VPS35 antibody

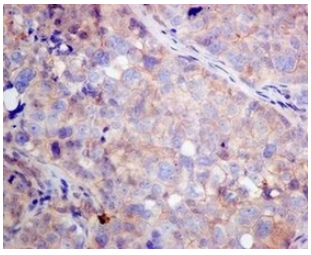
ab157220 staining VPS35 in wild-type HAP1 cells (top panel) and VPS35 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab157220 at 1/250 dilution and ab195889 at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VPS35 antibody [EPR11501(B)] (ab157220)

VPS35 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-VPS35 antibody

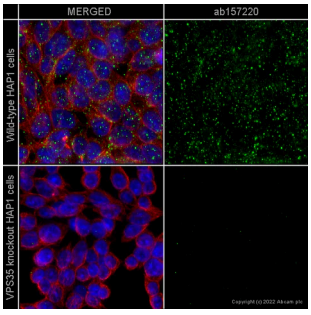
Immunohistochemical analysis of paraffin-embedded Human kidney tissue labeling VPS35 with ab157220 at 1/50 dilution.
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VPS35 antibody [EPR11501(B)] (ab157220)

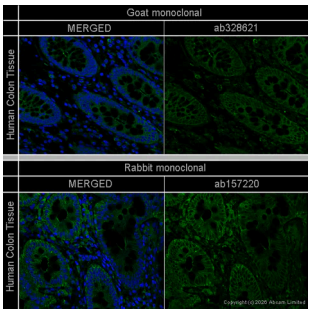
VPS35 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-VPS35 antibody

Immunohistochemical analysis of paraffin-embedded Human ovarian carcinoma tissue labeling VPS35 with ab157220 at 1/50 dilution.
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-VPS35 antibody [EPR11501(B)] (ab157220)

ab157220 staining VPS35 in wild-type HAP1 cells (top panel) and VPS35 knockout HAP1 cells (bottom panel). The cells were fixed with 100% Methanol (5 min), permeabilized with 0.1% Triton X-100 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 with ab157220 at 0.2 µg/mL and ab7291 at 1 µg/mL overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) (shown in pseudo colour green) and goat secondary antibody to Mouse IgG (Alexa Fluor® 594) (ab150120) (shown in pseudo colour red) both at 1/1000. Nuclear DNA was labelled with DAPI (shown in pseudo colour blue). Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VPS35 antibody [EPR11501(B)] (ab157220)

VPS35 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human Colon Tissue using rabbit Anti-VPS35 antibody

Immunohistochemical analysis of paraffin-embedded Human Colon Tissue tissue labeling VPS35 with ab328621 at 5 µg/ml dilution, followed by ab150133 Donkey Anti-Goat IgG H&L (Alexa Fluor® 488) preadsorbed at 1:1000 dilution.

Top two panels: Goat chimeric ab328621 [EPR11501(B)] shows specific endosomal staining of VPS35 in sections of formalin-fixed paraffin-embedded human colon. IHC staining performed on a Leica BOND.

The section was pre-treated using heat mediated antigen retrieval with EDTA (pH9.0) for 40 minutes. The section was then incubated at room temperature for 1 hour with ab328621 at 1/100 dilution, and then incubated for 1 hour with ab150133, Donkey Anti-Goat IgG H&L (Alexa Fluor®

488) preadsorbed at 1/1000 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Dako Fluorescence Mounting Medium®. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

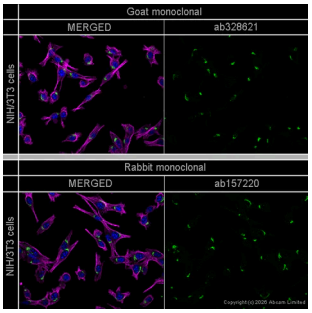
For other IHC staining systems (automated and non-automated), customers should optimize variable parameters such as antigen retrieval conditions, antibody concentrations and incubation times.

Bottom two panels: Side-by-side testing with the original rabbit version of the same clone, ab157220 (Anti-VPS35 antibody [EPR11501(B)]).

Counterstained with DAPI.

Secondary antibody only control: Secondary antibody is ab150133 Donkey Anti-Goat IgG H&L (Alexa Fluor® 488) preadsorbed at 1:1000 dilution.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 40 mins



Immunocytochemistry/ Immunofluorescence - Anti-VPS35 antibody [EPR11501(B)] (ab157220)

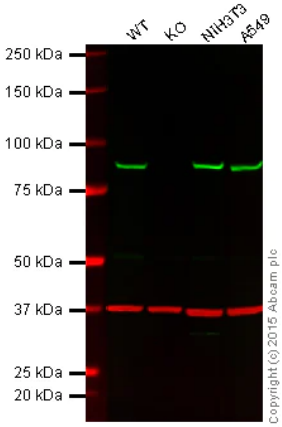
VPS35 Immunocytochemistry/ Immunofluorescence staining of NIH/3T3 using rabbit Anti-VPS35 antibody

Immunofluorescent analysis of 100% methanol-fixed, 0.1% TritonX-100 permeabilized NIH/3T3 cells labelling VPS35 with ab328621 at 1.0 µg/ml dilution , followed by ab150133 Donkey Anti-Goat IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green).

Top two panels: Goat chimeric ab328621 [EPR11501(B)] shows specific endosomal staining of VPS35 in NIH/3T3 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal donkey serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated for 2 hours at room temperature with ab328621 at 1 µg/ml (shown in green) and ab7291, Anti-alpha Tubulin antibody [DM1A] - Loading Control (shown in pseudocolour magenta). Cells were then incubated with ab150133, Donkey Anti-Goat IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution and ab150108, Donkey Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution. Nuclear DNA was labeled with DAPI (shown in blue). Also suitable in cells fixed with 4% paraformaldehyde (10 min). Image was acquired with a high-content analyser (Operetta CLS, Revvity) and a maximum intensity projection of confocal sections is shown. Bottom two panels: Side-by-side testing with the original rabbit version of the same clone, ab157220 (Anti-VPS35 antibody [EPR11501(B)]).

ab7291 Anti-alpha Tubulin mouse monoclonal antibody was used to counterstain tubulin at 1/1000 dilution (Magenta). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150133 Donkey Anti-Goat IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution.



Western blot - Anti-VPS35 antibody [EPR11501(B)] (ab157220)

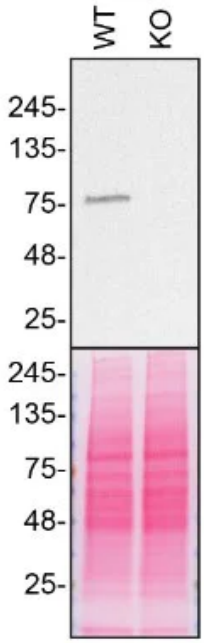
VPS35 Western blot staining using rabbit Anti-VPS35 antibody

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 - ab157220 observed at 91 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab157220 was shown to specifically react with VPS35 in wild-type HAP1 cells. No band was observed when VPS35 knockout samples were examined. Wild-type and VPS35 knockout samples were subjected to SDS-PAGE. ab157220 and ab8245 (loading control to GAPDH) were diluted 1/1000 and 1/2000 respectively 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.

All lanes:
Western blot - Anti-VPS35 antibody [EPR11501(B)] (ab157220)

Predicted band size: 92 kDa



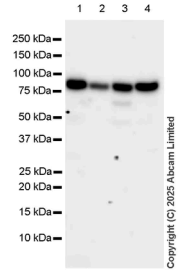
Western blot - Anti-VPS35 antibody [EPR11501(B)] (ab157220)

ab157220 was shown to react with VPS35 in wild-type HAP1 cells in Western blot with loss of signal observed in a VPS35 knockout cell line. Wild-type HAP1 and VPS35 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab157220 overnight at 4 °C at a 1/1000 dilution. Blots were incubated with secondary antibodies at 0.2 µg/mL before imaging.

These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.
All lanes:
Western blot - Anti-VPS35 antibody [EPR11501(B)] (ab157220) at 1/1000 dilution

Lane 1:
Wild-type HAP1 lysate at 20 µg

Lane 2:
VPS35 knock-out HAP1 lysate at 20 µg



Western blot - Anti-VPS35 antibody [EPR11501(B)] (ab157220)

VPS35 Western blot staining using rabbit Anti-VPS35 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
All lanes:
Western blot - Anti-VPS35 antibody [EPR11501(B)] (ab157220) at 1/1000 dilution

Lane 1:
Rat brain tissue lysate at 20 µg

Lane 2:
Rat heart tissue lysate at 20 µg

Lane 3:
Rat kidney tissue lysate at 20 µg

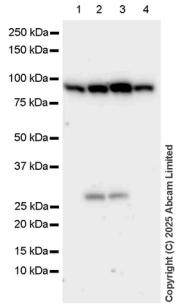
Lane 4:
Rat spleen tissue lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 92 kDa

Exposure time: 26s



Western blot - Anti-VPS35 antibody [EPR11501(B)] (ab157220)

VPS35 Western blot staining using rabbit Anti-VPS35 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
All lanes:
Western blot - Anti-VPS35 antibody [EPR11501(B)] (ab157220) at 1/1000 dilution

Lane 1:
C6 (rat glial tumor glial cell) whole cell lysate at 20 µg

Lane 2:
RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 3:
PC-12 (rat adrenal gland pheochromocytoma cell) whole cell lysate at 20 µg

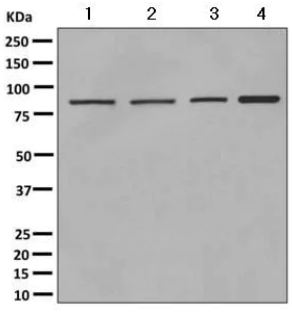
Lane 4:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 92 kDa

Exposure time: 48s



Western blot - Anti-VPS35 antibody [EPR11501(B)] (ab157220)

All lanes:
Western blot - Anti-VPS35 antibody [EPR11501(B)] (ab157220) at 1/10000 dilution

Lane 1:
HeLa cell lysate at 10 µg

Lane 2:
HepG2 cell lysate at 10 µg

Lane 3:
Raji cell lysate at 10 µg

Lane 4:
MOLT4 cell lysate at 10 µg

Secondary

All lanes:
Goat anti-rabbit HRP at 1/2000 dilution

Predicted band size: 92 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.