abcam

# ab57632

# Anti-VPS35 antibody [2D3]

KO Validated

## Key facts

| | |
|---|---|
| **Isotype** | IgG2a |
| **Host species** | Mouse |
| **Storage buffer** | pH: 7.4 |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | Recombinant Fragment Protein within Human VPS35 aa 650 to C-terminus. The exact immunogen used to generate this antibody is proprietary information. Database link Q96QK1 |
| **Clone number** | 2D3 |
| **Purity** | Tissue culture supernatant |
| **Light chain type** | kappa |
| **Purification notes** | Purified from TCS. |

## Reactivity data

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 0.2 µg/mL |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Recombinant fragment |
| **Dilution info** | - |
| **Notes** | - |

## Flow Cyt

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | ab170191 - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody. |

### Not recommended

| | |
|---|---|
| **Species** | Recombinant fragment |
| **Dilution info** | - |
| **Notes** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Recombinant fragment |
| **Dilution info** | - |
| **Notes** | This antibody has only been tested in WB against the recombinant fragment used as immunogen. We have no data on the detection of endogenous protein. |

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | This antibody has only been tested in WB against the recombinant fragment used as immunogen. We have no data on the detection of endogenous protein. |

## 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** | Blue Ice |
| **Appropriate short-term storage conditions** | +4°C |
| **Appropriate long-term storage conditions** | -20°C |
| **Aliquoting information** | Upon delivery aliquot |
| **Storage information** | Avoid freeze / thaw cycle |

## Notes

This product was changed from ascites to tissue culture supernatant on 05 Feb 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.

## 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.

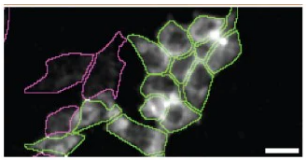
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We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

## 8 product images

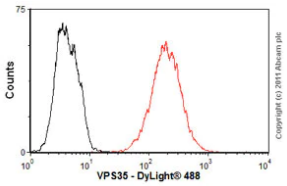


### Immunocytochemistry/ Immunofluorescence - Anti-VPS35 antibody [2D3] (ab57632)

VPS35 Immunocytochemistry/ Immunofluorescence staining of HAP1 cells using mouse Anti-VPS35 antibody

ab57632 was shown to react with VPS35 in wild-type HAP1 cells in immunocytochemistry with loss of signal observed in a VPS35 knockout cell line. Wild-type and knockout cells were mixed and pelleted at a 1:1 ratio on coverslips. The cells were fixed with 4% paraformaldehyde (15 min) then permeabilized with 0.1% Triton X-100 (10min) and then blocked with 1x PBS, 0.01% Triton X-100, 5% BSA, 5% NGS. The cells were then incubated with ab57632 at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat anti-rabbit secondary antibody to (Alexa Fluor® 555) at 0.5 µg/ml. Acquisition of the green (wild-type), red (antibody staining) and far-red (knockout) channels was performed. Representative grayscale images of the red channel are shown. Wild-type and knockout cells are outlined with yellow and magenta dashed line, respectively. Schematic representation of the mosaic strategy used is shown on the bottom-right panel. Image was acquired with a Zeiss(LSM-880).

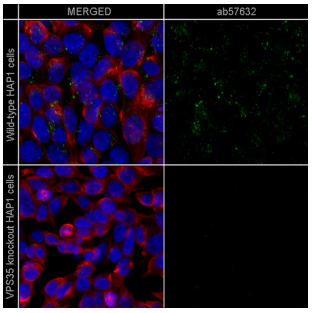
These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.




## Flow Cytometry - Anti-VPS35 antibody [2D3] (ab57632)

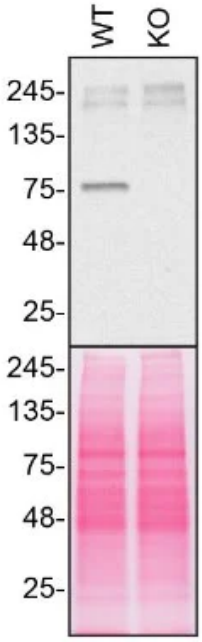
VPS35 Flow Cytometry staining using mouse Anti-VPS35 antibody

Overlay histogram showing HEK293 cells stained with ab57632 (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 (ab57632, 0.5µg/1x$10^6$ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (ab91361, 2µg/1x$10^6$ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HEK293 cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.
**This image was generated using a version of the antibody produced in ascites.**




## Immunocytochemistry/ Immunofluorescence - Anti-VPS35 antibody [2D3] (ab57632)

ab57632 staining VPS35 in wild-type HAP1 cells (top panel) and VPS35 knockout HAP1 cells (bottom panel). The cells were fixed with 4% PFA (10 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 ab57632 at 0.2 µg/mL and ab6046 at 1 µg/mL overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Mouse IgG (Alexa Fluor® 488) (ab150117) (shown in pseudo colour green) and goat secondary antibody to Rabbit IgG (Alexa Fluor® 594) (ab150084) (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.



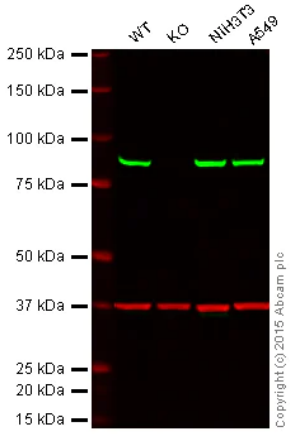

## Western blot - Anti-VPS35 antibody [2D3] (ab57632)

ab57632 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 ab57632 overnight at 4 °C at a 1/270 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 [2D3] (ab57632) at 1/270 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 [2D3] (ab57632)

VPS35 Western blot staining using mouse Anti-VPS35 antibody

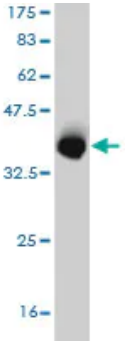
**Lane 1**: Wild-type HAP1 cell lysate (20 µg)
**Lane 2**: VPS35 knockout HAP1 cell lysate (20 µg)
**Lane 3**: NIH/3T3 cell lysate (20 µg)
**Lane 4**: A549 cell lysate (20 µg)
**Lanes 1 - 4**: Merged signal (red and green). Green - ab57632 observed at 91 kDa. Red - loading control, ab181602, observed at 37 kDa.

ab57632 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. ab5732 and ab181602 (loading control to GAPDH) were diluted 1µg/mL and 1/10,000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.

**This image was generated using a version of the antibody produced in ascites.**

All lanes:
Western blot - Anti-VPS35 antibody [2D3] (ab57632)

Predicted band size: 92 kDa




## Western blot - Anti-VPS35 antibody [2D3] (ab57632)

Western blot against tagged recombinant protein immunogen using ab57632 VPS35 antibody at 1 µg/ml. Predicted band size of immunogen is 37 kDa
**This image was generated using a version of the antibody produced in ascites.**

All lanes:
Western blot - Anti-VPS35 antibody [2D3] (ab57632)

Predicted band size: 92 kDa

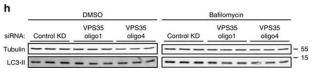



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## Western blot - Anti-VPS35 antibody [2D3] (ab57632)

VPS35 Western blot staining using mouse Anti-VPS35 antibody

Western Blotting using Anti-VPS35 antibody [2D3], ab57632. Publication image from Seaman, M. N. et al., 2014, Nat Commun, 24819384. Legend direct from paper.

VPS35 D620N impairs autophagy, while VPS35 knockdown has only modest effects.(a) HeLa cells stably expressing GFP-VPS35 WT and D620N were treated with bafilomycin A1 or DMSO vehicle control. Endogenous LC3-II and tubulin levels were examined by western blot. A representative experiment of six experiments is shown. (b) Quantification of the representative experiment in triplicate shown in a, in which endogenous LC3-II levels are normalized to tubulin and expressed as a ratio of levels in WT. ***P=7.86 x 10−6 (DMSO) and 3.81 x 10−5 (Baf) by 2-tailed Student’s t-test. (c) HeLa cells stably expressing GFP-VPS35 WT and D620N were transfected with mRFP-LC3. The number of LC3 vesicles was quantified by Cellomics automated fluorescence microscopy. A representative experiment of three independent experiments is shown, with 344 (WT) and 401 (D620N) cells analysed. ***P<0.0001 by 2-tailed Student’s t-test. (d) GFP-VPS35 WT and D620N-expressing cells were transfected with HA-Q74 and immunostained for HA. The percentage of transfected cells with aggregates was counted by a blinded experimenter. The quantification

shows the mean of three experiments in triplicate with minimum 200 cells per replicate. ***P=0.00086 by 1-tailed Student’ s t-test. (e) Confocal images representative of the experiment described in d. Scale bar, 20 µm. (f) Cells stably expressing WT and D620N GFP-VPS35 were transfected with GFP-α-synuclein A53T and GFP for 48 h and analyzed by western blotting. (g) Quantification of the representative experiment in triplicate in f, in which the level ofα-synuclein was expressed as a ratio to GFP. A representative experiment of two independent experiments is shown. **P=0.0047 by 2-tailed Student’ s t-test. (h) VPS35 was knocked down with two individual siRNA nucleotides in HeLa cells, and cells were treated with bafilomycin A1 and lysed as in a. A representative experiment is shown in triplicate. (i) Quantification of three independent experiments in triplicate. *P=0.026; other results non-significant by 2-tailed Student’ s t-test. (j) Protein levels of CSC, including VPS35, VPS26 and VPS29), were assessed upon VPS35 knockdown, confirming previous results that knockdown of one component destabilizes the CSC. All error bars indicate s.e.m.

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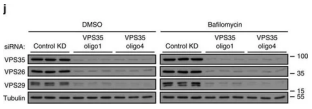



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