abcam

ab45169

Anti-Huntingtin antibody [EP867Y]

Recombinant | RabMAb | KO Validated | 20ul selling size

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	EP867Y
Purification technique	Affinity purification Protein A
Concentration	1.53 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Expected

Species	Mouse
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Predicted

Species	Rat
Dilution info	-
Notes	-

IP

Not recommended

Species	Mouse, Human, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/10000
Notes	-

Expected

Species	Mouse
Dilution info	1/10000
Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/50 - 1/100
Notes	-

Expected

Species	Mouse
Dilution info	1/50 - 1/100
Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

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

Expected

Species	Mouse

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

Predicted

Species	Rat
Dilution info	-
Notes	-

Target data

See full target information HTT

Function	Huntingtin. May play a role in microtubule-mediated transport or vesicle function. Huntingtin, myristoylated N-terminal fragment. Promotes the formation of autophagic vesicles.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
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

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

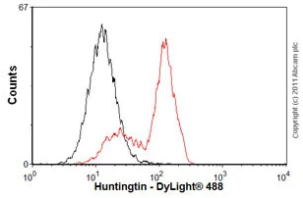
We do not recommend this combination. It is not covered by our product promise.

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.

9 product images

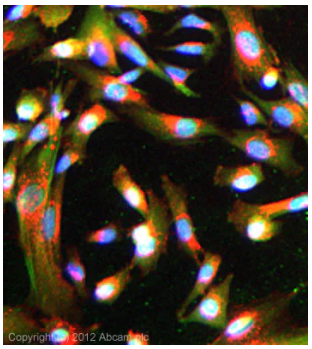


Flow Cytometry (Intracellular) - Anti-Huntingtin antibody [EP867Y] (ab45169)

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

Overlay histogram showing SH-SY5Y cells stained with ab45169 (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 (ab45169, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody

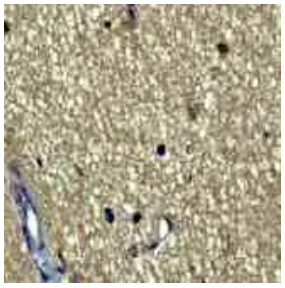
gave a positive signal in SH-SY5Y cells fixed with 4% paraformaldehyde/permeabilized in 0.1% PBS-Tween used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-Huntingtin antibody [EP867Y] (ab45169)

Huntingtin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Huntingtin antibody

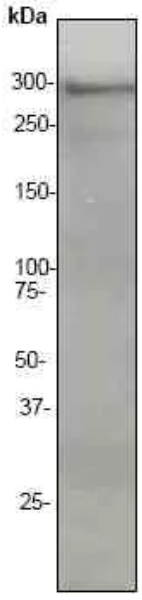
ICC/IF image of ab45169 stained SKNSH cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab45169, 1/200 dilution) overnight at +4°C. The secondary antibody (green) was ab96899, DyLight® 488 goat anti-rabbit 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-Huntingtin antibody [EP867Y] (ab45169)

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

ab45169 staining human Huntingtin in human brain tissue by immunohistochemistry using paraffin embedded tissue.
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



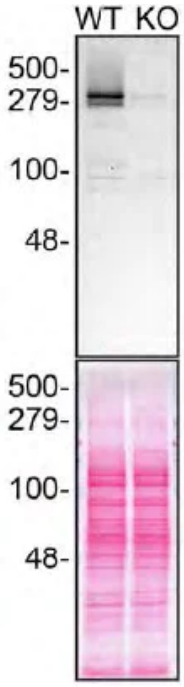
Western blot - Anti-Huntingtin antibody [EP867Y] (ab45169)

All lanes:
Western blot - Anti-Huntingtin antibody [EP867Y] (ab45169) at 1/10000 dilution

All lanes:
SH-SY-5Y cell lysate

Predicted band size: 348 kDa

Observed band size: 300 kDa



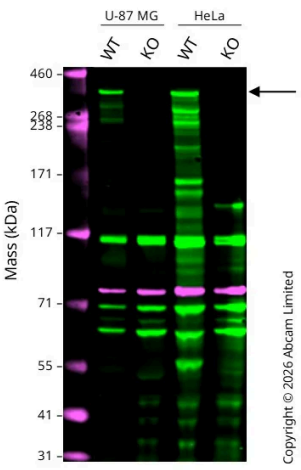
Western blot - Anti-Huntingtin antibody [EP867Y] (ab45169)

ab45169 was shown to react with HTT in wild-type DMS53 cells in Western blot with loss of signal observed in a HTT knockout cell line. Wild-type DMS53 and HTT knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab45169 overnight at 4 °C at a 1/5000 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-Huntingtin antibody [EP867Y] (ab45169) at 1/5000 dilution

Lane 1:
Wild-type DMS53 lysate at 30 µg

Lane 2:
HTT knock-out DMS53 lysate at 30 µg



Western blot - Anti-Huntingtin antibody [EP867Y] (ab45169)

Huntingtin Western blot staining using rabbit Anti-Huntingtin antibody

Western blot: Rabbit Monoclonal [EP867Y] to Huntingtin ab45169 staining at 1/1000 dilution, shown in green; Mouse anti-CANX ab238078 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 348 kDa in Wild-type U-87 MG cell lysates with no signal observed at this size in HTT knockout U-87 MG cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-Huntingtin antibody [EP867Y] (ab45169) at 1/1000 dilution

Lane 1:
Wild-type U-87 MG cell lysates at 20 µg

Lane 2:
Western blot - Human HTT knockout U-87 MG cell line (ab306706) at 20 µg

Lane 3:
Wild-type HeLa cell lysates at 20 µg

Lane 4:
Western blot - Human HTT (Huntingtin) knockout HeLa cell lysate (ab256946) at 20 µg

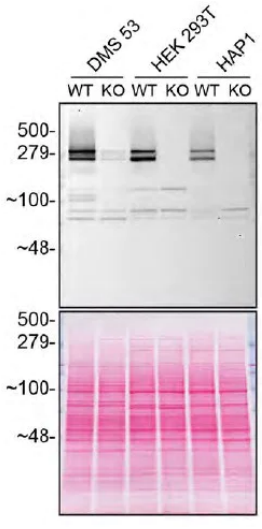
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 348 kDa

Observed band size: 348 kDa



Western blot - Anti-Huntingtin antibody [EP867Y] (ab45169)

ab45169 was shown to react with HTT in wild-type DMS53, HEK 293T and HAP1 cells in Western blot with loss of signal observed in a HTT knockout cell lines. Wild-type and HTT knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab45169 overnight at 4 °C at a 1/5000 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-Huntingtin antibody [EP867Y] (ab45169) at 1/5000 dilution

Lane 1:
Wild-type DMS53 lysate at 30 µg

Lane 2:

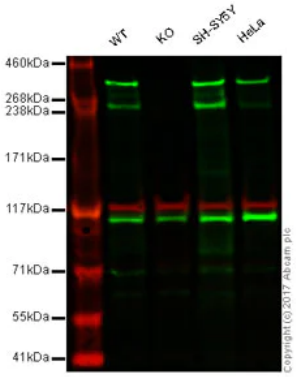
HTT knock-out DMS53 lysate at 30 µg

Lane 3:
Wild-type HEK 293T lysate at 30 µg

Lane 4:
HTT knock-out HEK 293T lysate at 30 µg

Lane 5:
Wild-type HAP1 lysate at 30 µg

Lane 6:
HTT knock-out HAP1 lysate at 30 µg



Western blot - Anti-Huntingtin antibody [EP867Y] (ab45169)

Huntingtin Western blot staining using rabbit Anti-Huntingtin antibody

Lanes 1 - 4: Merged signal (red and green). Green - ab45169 observed at 348 kDa. Red - loading control, ab18058, observed at 130 kDa.
ab45169 was shown to specifically recognize Huntingtin in wild-type HAP1 cells along with additional cross reactive bands. No band was observed when Huntingtin knockout samples were examined. Wild-type and Huntingtin knockout samples were subjected to SDS-PAGE. ab45169 and ab18058 (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/10,000 dilution and 1/10,000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed ab216777 secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Huntingtin antibody [EP867Y] (ab45169) at 1/10000 dilution

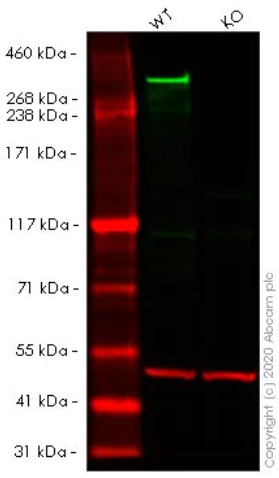
Lane 1:
Wild-type HAP1 whole cell lysate at 20 µg

Lane 2:
Huntingtin knockout HAP1 whole cell lysate at 20 µg

Lane 3:
SH-SY5Y whole cell lysate at 20 µg

Lane 4:
HeLa whole cell lysate at 20 µg

Predicted band size: 348 kDa



Western blot - Anti-Huntingtin antibody [EP867Y] (ab45169)

Huntingtin Western blot staining using rabbit Anti-Huntingtin antibody

Lanes 1- 2: Merged signal (red and green). Green - ab45169 observed at 348 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) observed at 50 kDa.
ab45169 was shown to react with Huntingtin in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265976 (knockout cell lysate ab256946) was used. Wild-type HeLa and HTT knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab45169 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Huntingtin antibody [EP867Y] (ab45169) at 1/10000 dilution

Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
HTT knockout HeLa cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 348 kDa

Observed band size: 348 kDa

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