abcam

ab225573

Anti-Huntingtin antibody [EP867Y] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EP867Y
Purification technique	Affinity purification Protein A
Concentration	1.041 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-

Notes	-

Expected

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

Predicted

Species	Rat
Dilution info	-
Notes	-

IP

Not recommended

Species	Mouse, Human, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human, Mouse
Dilution info	-
Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

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

Predicted

Species	Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376- Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse
Dilution info	-
Notes	ab199376- 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	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab225573 is the carrier-free version of ab45169.

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.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

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.

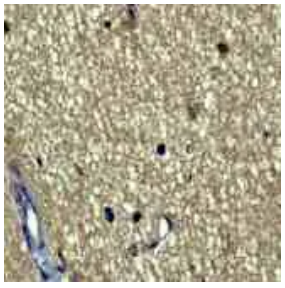
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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8 product images

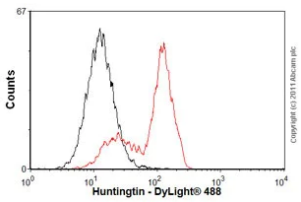


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Huntingtin antibody [EP867Y] - BSA and Azide free (ab225573)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab45169).

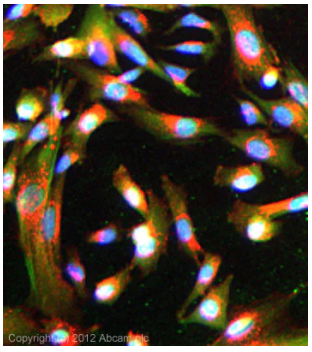
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-Huntingtin antibody [EP867Y] - BSA and Azide free (ab225573)

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

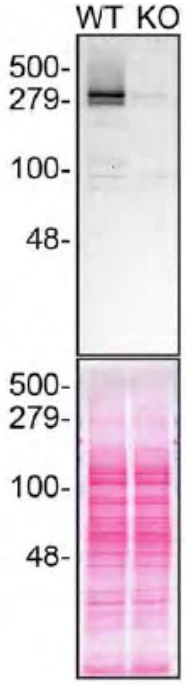
Overlay histogram showing SH-SY5Y cells stained with ab45169 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions 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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab45169).



Immunocytochemistry/ Immunofluorescence - Anti-Huntingtin antibody [EP867Y] - BSA and Azide free (ab225573)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab45169).



Western blot - Anti-Huntingtin antibody [EP867Y] - BSA and Azide free (ab225573)

This data was developed using ab45169, the same antibody clone in a different buffer formulation.

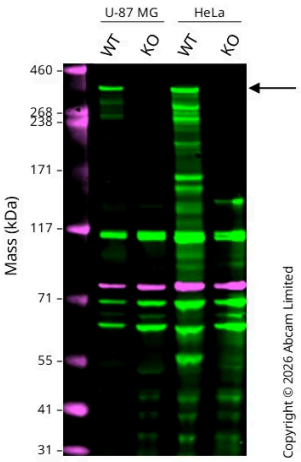
ab45169 was shown to react with HTT in wild-type DMS53 cells in Western blot with loss of signal observed in a HTT knockout cell line. Wild-type DMS53 and HTT knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab45169 overnight 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] - BSA and Azide free (ab225573)

Huntingtin Western blot staining using rabbit Anti-Huntingtin antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab45169).

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

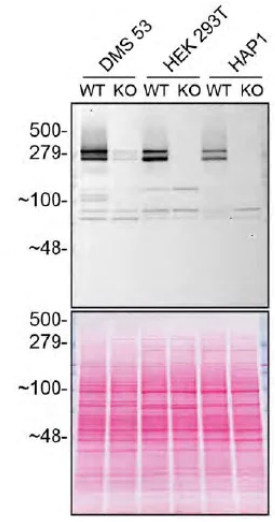
Secondary

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] - BSA and Azide free (ab225573)

This data was developed using ab45169, the same antibody clone in a different buffer formulation.

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

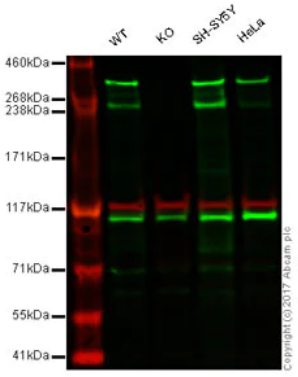
Lane 2:
HTT knock-out DMS53 lysate at 30 µg

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] - BSA and Azide free (ab225573)

Huntingtin Western blot staining using rabbit Anti-Huntingtin antibody

This WB data was generated using the same anti-Huntingtin antibody clone [EP867Y] in a different buffer formulation (cat# ab45169).
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 recognize Huntingtin when Huntingtin knockout samples were used, along with additional cross-reactive bands. 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 both at 1/10000 dilution. 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 1/10000 dilution for 1 hour at room temperature before imaging.

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

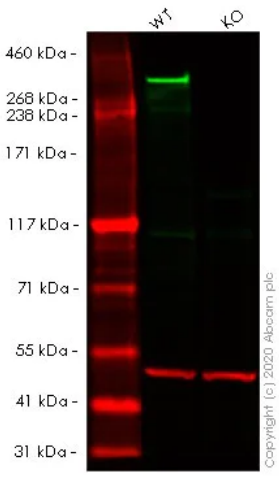
Lane 1:
Wild-type HAP1 whole cell lysate at 20 µg

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] - BSA and Azide free (ab225573)

Huntingtin Western blot staining using rabbit Anti-Huntingtin antibody

This data was developed using the same antibody clone in a different buffer formulation (ab45169).
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'.