## Overview

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>Product name</strong></td>
<td>Anti-Huntingtin antibody [EPR5526]</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td>Rabbit monoclonal [EPR5526] to Huntingtin</td>
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<tr>
<td><strong>Host species</strong></td>
<td>Rabbit</td>
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<tr>
<td><strong>Tested applications</strong></td>
<td>Suitable for: Flow Cyt, IHC-FoFr, ICC/IF, WB, IHC-P</td>
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<td><strong>Species reactivity</strong></td>
<td>Reacts with: Mouse, Rat, Human</td>
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<td><strong>Immunogen</strong></td>
<td>Synthetic peptide within Human Huntingtin aa 1-100. The exact sequence is proprietary. Database link: P42858</td>
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<td><strong>Positive control</strong></td>
<td>WB: SH-SY5Y, HeLa, HAP1, PC-12 and Neuro-2a whole cell lysates; Mouse and rat brain lysates. IHC-P: Human cerebral cortex and astrocytoma tissue; Mouse and rat testis tissue. ICC/IF: Neuro-2a and SH-SY5Y cell lines. Flow Cyt: SH-SY5Y cell line. IHC-Fr: Mouse and rat cerebrum.</td>
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<td><strong>General notes</strong></td>
<td>This product is a recombinant monoclonal antibody, which offers several advantages including:</td>
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<td>- High batch-to-batch consistency and reproducibility</td>
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<td></td>
<td>- Improved sensitivity and specificity</td>
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<td>- Long-term security of supply</td>
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<td>- Animal-free production</td>
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<td>For more information see here.</td>
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Our RabMab® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

Reproducibility is key to advancing scientific discovery and accelerating scientists’ next breakthrough.

Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.

We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™.
guarantee. In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

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, as well as customer reviews and Q&As.

Properties

Form
Liquid

Storage instructions

Storage buffer
pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: 40% Glycerol (glycerin, glycerine), 59% PBS, 0.05% BSA

Purity
Protein A purified

Clonality
Monoclonal

Clone number
EPR5526

Isotype
IgG

Applications

Our Abpromise guarantee covers the use of ab109115 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tr>
<td>Flow Cyt</td>
<td>1/250, ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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<tr>
<td>IHC-FoFr</td>
<td>1/100, Perform Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).</td>
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<tr>
<td>ICC/IF</td>
<td>1/1000.</td>
<td></td>
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<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐ 1/5000. Detects a band of approximately 348 kDa (predicted molecular weight: 348 kDa).</td>
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<tr>
<td>IHC-P</td>
<td>1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.</td>
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### Function
May play a role in microtubule-mediated transport or vesicle function.

### Tissue specificity
Expressed in the brain cortex (at protein level). Widely expressed with the highest level of expression in the brain (nerve fibers, varicosities, and nerve endings). In the brain, the regions where it can be mainly found are the cerebellar cortex, the neocortex, the striatum, and the hippocampal formation.

### Involvement in disease
Defects in HTT are the cause of Huntington disease (HD) [MIM:143100]. HD is an autosomal dominant neurodegenerative disorder characterized by involuntary movements (chorea), general motor impairment, psychiatric disorders and dementia. Onset of the disease occurs usually in the third or fourth decade of life and symptoms progressively worsen leading to death in 10 to 20 years. Onset and clinical course depend on the degree of poly-Gln repeat expansion, longer expansions resulting in earlier onset and more severe clinical manifestations. HD affects 1 in 10,000 individuals of European origin. Neuropathology of Huntington disease displays a distinctive pattern with loss of neurons, especially in the caudate and putamen (striatum).

### Sequence similarities
Belongs to the huntingtin family. Contains 10 HEAT repeats.

### Domain
The N-terminal Gln-rich and Pro-rich domain has great conformational flexibility and is likely to exist in a fluctuating equilibrium of alpha-helical, random coil, and extended conformations.

### Post-translational modifications
Cleaved by apopain downstream of the polyglutamine stretch. The resulting N-terminal fragment is cytotoxic and provokes apoptosis. Forms with expanded polyglutamine expansion are specifically ubiquitinated by SYVN1, which promotes their proteasomal degradation.

### Cellular localization

### Images

**Western blot - Anti-Huntingtin antibody [EPR5526] (ab109115)**

![Western blot](image)

**All lanes**: Anti-Huntingtin antibody [EPR5526] (ab109115) at 1/10000 dilution

**Lane 1**: Wild-type HeLa cell lysate

**Lane 2**: HTT knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size**: 348 kDa

**Observed band size**: 348 kDa

**Lanes 1-2**: Merged signal (red and green). Green - ab109115 observed at 348 kDa. Red - Anti-alpha Tubulin antibody [DM1A] -
Loading Control (ab7291) observed at 50 kDa.

ab109115 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. ab109115 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.

Immunohistochemistry (Frozen) analysis of mouse cerebellum tissue sections labeling Huntingtin with purified ab109115 at 1/100 (13.4 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/1000 (2 µg/ml) was used as the secondary antibody. Sections were fixed with 4% paraformaldehyde and permeabilised with 0.2% Triton X-100. Negative control: PBS instead of the primary antibody. DAPI (blue) was used as nuclear counterstain. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20) was performed.
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Neuro-2a (Mouse neuroblastoma cells) cells labeling Huntingtin with purified ab109115 at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear and cytoplasmic staining on Neuro-2a cell line.

The nuclear counter stain is DAPI (blue).

Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:
1. ab191472 at 1/1000 dilution followed by ab150120 (Alexa Fluor® 594 Goat anti-Mouse secondary) at 1/1000 dilution.
2. ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor® 488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.

**All lanes**: Anti-Huntingtin antibody [EPR5526] (ab109115) at 1/10000 dilution

- **Lane 1**: Wild-type HAP1 whole cell lysate
- **Lane 2**: HTT knockout HAP1 whole cell lysate
- **Lane 3**: SH-SY5Y whole cell lysate
- **Lane 4**: HeLa whole cell lysate

Lysates/proteins at 20 µg per lane.

**Predicted band size**: 348 kDa

**Lanes 1 - 4**: Merged signal (red and green). Green - ab109115 observed at 348 kDa. Red - loading control, ab18058, observed at 130 kDa.

ab109115 was shown to specifically recognize HTT in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when HTT knockout samples were examined. Wild-type and HTT knockout samples were subjected to SDS-PAGE. Unpurified ab109115 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-Mouse IgG H&L (IRDye® 680RD) preabsorbed 
ab216776 secondary antibodies at 1/10,000 dilution for 1 hour at 
room temperature before imaging.

Immunohistochemical analysis of paraffin-embedded Human 
astrocytoma labeling Huntingtin with purified ab109115 at 1/100 
dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 
1/500. Counter stained with Hematoxylin. Nuclear staining on 
cancer cells of astrocytoma.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 
9.0 before commencing with IHC staining protocol.

Immunohistochemistry (Frozen) analysis of mouse cerebrum tissue 
sections labeling Huntingtin with purified ab109115 at 1/100 (13.4 
µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 
1/1000 (2 µg/ml) was used as the secondary antibody. Sections 
were fixed with 4% paraformaldehyde and permeabilised with 0.2% 
Triton X-100. Negative control: PBS instead of the primary 
antibody. DAPI (blue) was used as nuclear counterstain. Heat 
mediated antigen retrieval using sodium citrate buffer (10mM citrate 
pH 6.0 + 0.05% Tween-20) was performed.
**All lanes**: Anti-Huntingtin antibody [EPR5526] (ab109115) at 1/5000 dilution (purified)

**Lane 1**: SH-SY5Y (Human neuroblastoma from bone marrow cells) whole cell lysate

**Lane 2**: HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

**Lane 3**: PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate

**Lane 4**: Neuro-2a (Mouse neuroblastoma cells) whole cell lysate

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution

**Predicted band size**: 348 kDa

**Observed band size**: 348 kDa

**Exposure time**: 1 second

Blocking and Diluting buffer and concentration: 5% NFDM /TBST
Immunohistochemical analysis of paraffin-embedded Human cerebral cortex tissue labeling Huntingtin with purified ab109115 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500. Counter stained with Hematoxylin. Nuclear staining on neuron of human cerebral cortex was observed.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

All lanes: Anti-Huntingtin antibody [EPR5526] (ab109115) at 1/50000 dilution (purified)

Lane 1: Mouse brain lysate
Lane 2: Rat brain lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution

Predicted band size: 348 kDa
Observed band size: 348 kDa

Exposure time: 30 seconds

Blocking and Diluting buffer and concentration: 5% NFDM /TBST
Flow cytometric analysis of 4% paraformaldehyde-fixed SH-SY5Y (Human neuroblastoma from bone marrow cells) cells labeling Huntingtin with purified ab109115 at 1/250 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/500 dilution was used as the secondary antibody.

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized SH-SY5Y (Human neuroblastoma from bone marrow cells) cells labeling Huntingtin with purified ab109115 at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear and cytoplasmic staining on SH-SY5Y cell line.

The nuclear counter stain is DAPI (blue).

Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (Alexa Fluor® 594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:
1. ab191472 at 1/1000 dilution followed by ab150120 (Alexa Fluor® 594 Goat anti-Mouse secondary) at 1/1000 dilution.
2. ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor® 488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Huntingtin antibody [EPR5526] (ab109115)

Immunohistochemical analysis of paraffin-embedded Mouse testis labeling Huntingtin with purified ab109115 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500. Counter stained with Hematoxylin. Cytoplasmic staining on spermatogenic cells of mouse testis.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Huntingtin antibody [EPR5526] (ab109115)

Immunohistochemical analysis of paraffin-embedded Rat testis labeling Huntingtin with purified ab109115 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500. Counter stained with Hematoxylin. Weak cytoplasmic staining on spermatogenic cells of rat testis.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Anti-Huntingtin antibody [EPR5526] (ab109115)

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