abcam

Product datasheet

Anti-Huntingtin antibody [EPR5526] - BSA and Azide free ab209668

KO VALIDATED Recombinant RabMAb

1 References 12 Images

Overview

Product name	Anti-Huntingtin antibody [EPR5526] - BSA and Azide free
Description	Rabbit monoclonal [EPR5526] to Huntingtin - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IHC-FoFr, IHC-P, WB, ICC/IF **Unsuitable for:** Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: SH-SY5Y, HeLa, HAP1, PC-12 and Neuro-2a whole cell lysates and mouse and rat brain lysates. IHC-P: Human cerebral cortex tissue, Human astrocytoma tissue, Mouse testis and Rat testis tissue. ICC/IF: Neuro-2a, SH-SY5Y and HeLa cell lines. IHC-Fr: Mouse cerebellum tissue
General notes	ab209668 is the carrier-free version of **ab109115**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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. 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 production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR5526
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab209668 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-FoFr		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 348 kDa (predicted molecular weight: 348 kDa).
ICC/IF		Use at an assay dependent concentration.

Application notes Is unsuitable for Flow Cyt (Intra).

Target

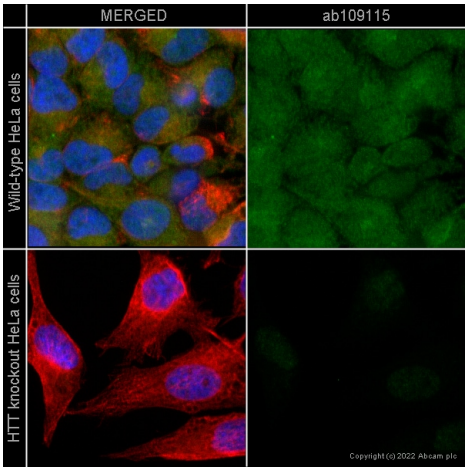
Function May play a role in microtubule-mediated transport or vesicle function.

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	Cytoplasm. Nucleus. The mutant Huntingtin protein colocalizes with AKAP8L in the nuclear matrix of Huntington's disease neurons.

Images

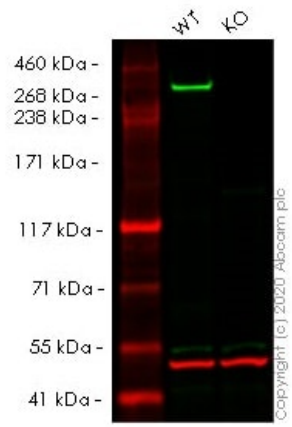


Immunocytochemistry/ Immunofluorescence - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

ab109115 staining HTT in wild-type HeLa cells (top panel) and HTT knockout HeLa cells (bottom panel, available as **ab265976**). The cells were fixed with 4% formaldehyde (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 **ab109115** at 1.0 µg/mL and **ab7291** at 1.0 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 green) and goat secondary antibody to Mouse IgG (Alexa Fluor® 594) (**ab150120**) (shown in red) both at 1/1000. Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

This data was developed using the same antibody clone in a different buffer formulation (**ab109115**).



Western blot - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

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.

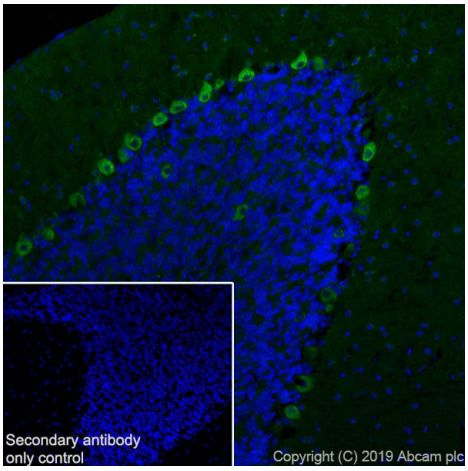
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab109115**).

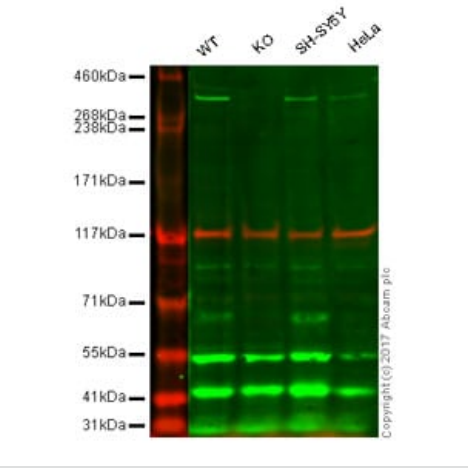
Lanes 1- 2: Merged signal (red and green). Green - **ab109115** observed at 348 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

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 (PFA perfusion fixed frozen sections) - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109115**).



Western blot - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

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.

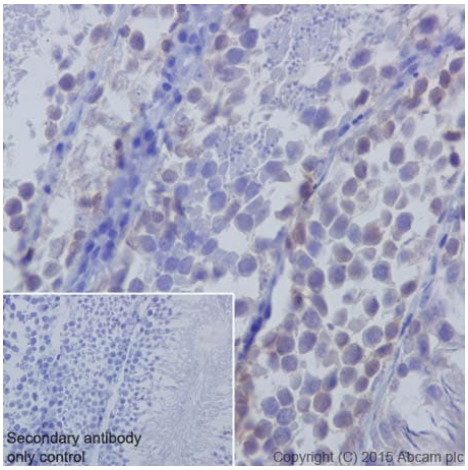
Predicted band size: 348 kDa

This WB data was generated using the same anti-Huntingtin antibody clone, EPR5526, in a different buffer formulation (cat# **ab109115**).

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 exmined. 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.

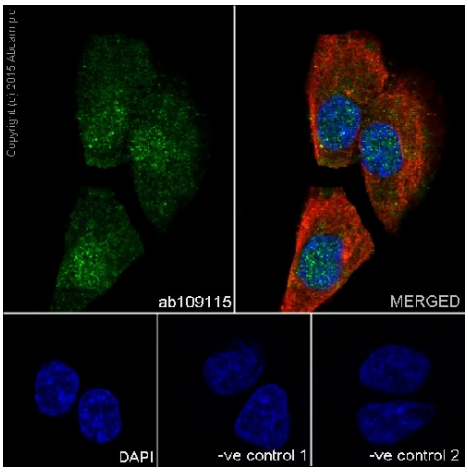


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

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.

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109115**).



Immunocytochemistry/ Immunofluorescence - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

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).

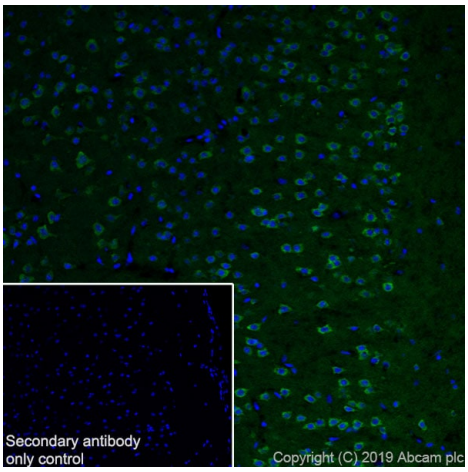
Confocal image showing nuclear and cytoplasmic staining on SH-SY5Y cell line.

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).

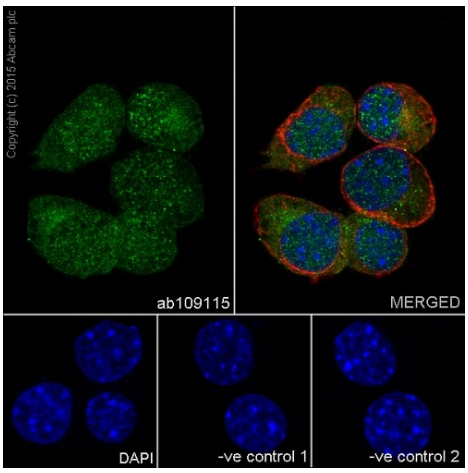
The negative controls are as follows:
1. **ab191472** at 1/1000 dilution followed by **ab150120** (Alexa Fluor® 594 Goat anti-Mouse secondary) at 1/1000 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor® 488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109115**).



Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide **ab109115**).



Immunocytochemistry/ Immunofluorescence - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

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).

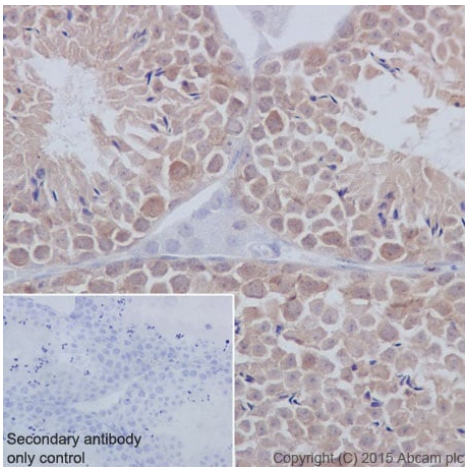
Confocal image showing nuclear and cytoplasmic staining on Neuro-2a cell line.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109115**).

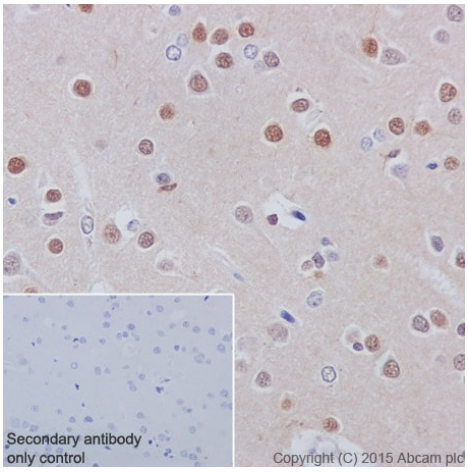


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

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.

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109115**).

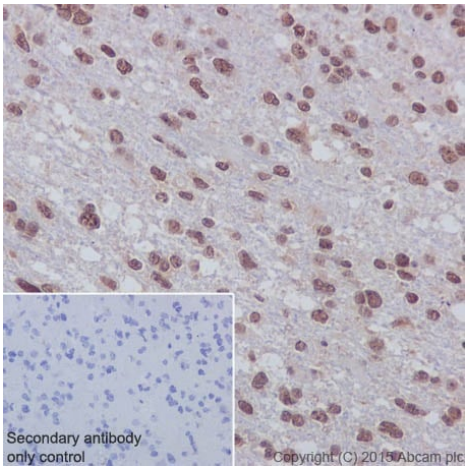


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

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.

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109115**).

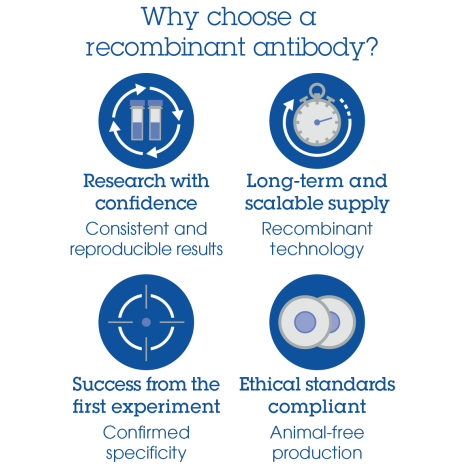


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

This IHC data was generated using the same anti-Huntingtin antibody clone, EPR5526, in a different buffer formulation (cat# **ab109115**).

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.

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours

- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors