abcam

Product datasheet

Anti-Huntingtin antibody [EPR5526] ab109115

敲除验证 重组 RabMAb

4 Abreviews 31 References 15 图像

概述

产品名称	Anti-Huntingtin**抗体**[EPR5526]
描述	**兔单克隆抗体**[EPR5526] to Huntingtin
宿主	Rabbit
经测试应用	**适用于:** IHC-FoFr, ICC/IF, WB, IHC-P **不适用于:** Flow Cyt (Intra)
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	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, SH-SY5Y and HeLa cell lines. IHC-Fr: Mouse and rat cerebrum.
常规说明	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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 59% PBS, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR5526

同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用**ab109115**于以下的**经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

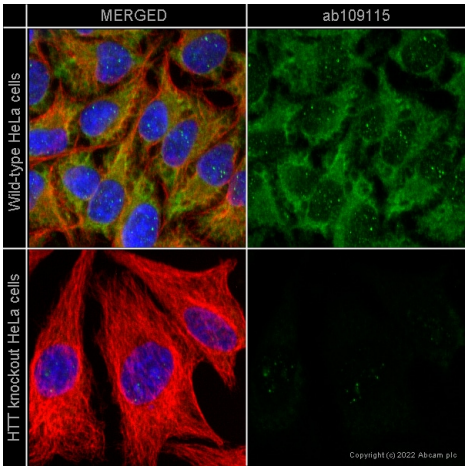
应用	Ab评论	说明
IHC-FoFr		1/100. Perform Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).
ICC/IF		Use a concentration of 1 µg/ml.
WB	★★★★★ (3)	1/5000. Detects a band of approximately 348 kDa (predicted molecular weight: 348 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

应用说明 Is unsuitable for Flow Cyt (Intra).

靶标

功能	May play a role in microtubule-mediated transport or vesicle function.
组织特异性	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.
疾病相关	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).
序列相似性	Belongs to the huntingtin family. Contains 10 HEAT repeats.
结构域	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.
翻译后修饰	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.
细胞定位	Cytoplasm. Nucleus. The mutant Huntingtin protein colocalizes with AKAP8L in the nuclear matrix of Huntington's disease neurons.

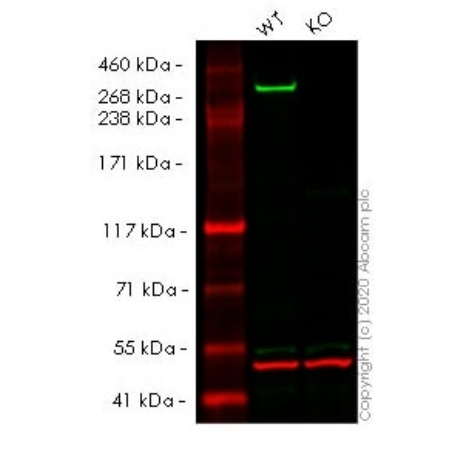
图片



Immunocytochemistry/ Immunofluorescence - Anti-Huntingtin antibody [EPR5526] (ab109115)

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 100% Methanol (5 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.



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

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.

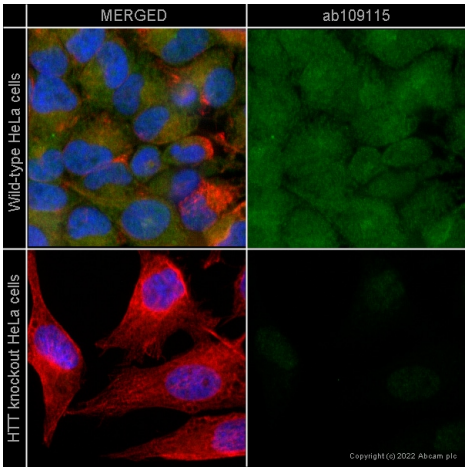
Performed under reducing conditions.

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

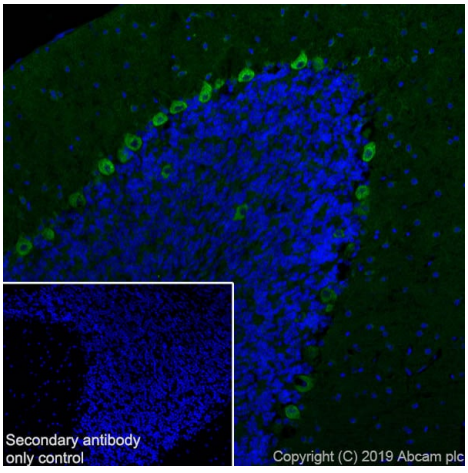
and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) overnight at 4°C at a 1 in 10000 Dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Huntingtin antibody [EPR5526] (ab109115)

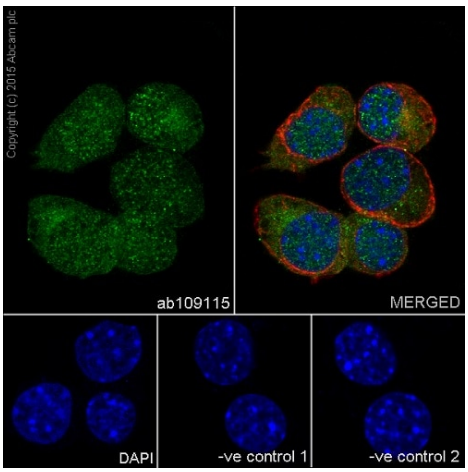
ab109115 staining HTT in wild-type HeLa cells (top panel) and HTT knockout HeLa cells (bottom panel, available as **ab265976**). The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% 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.



Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-Huntingtin antibody [EPR5526] (ab109115)

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.



Immunocytochemistry/ Immunofluorescence - Anti-Huntingtin antibody [EPR5526] (ab109115)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized

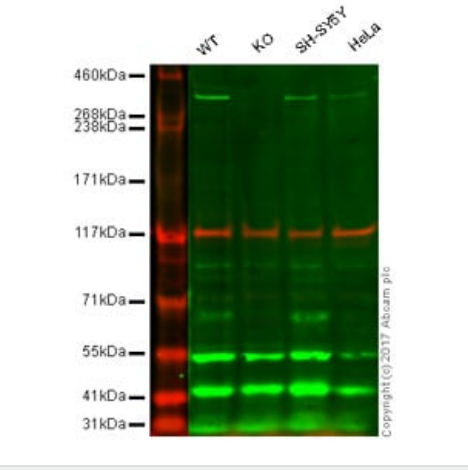
Neuro-2a (Mouse neuroblastoma cells) cells labeling Huntingtin with purified ab109115 at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

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.



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

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

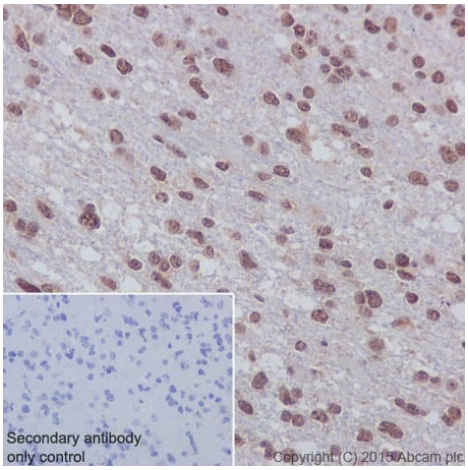
Lysates/proteins at 20 µg per lane.

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

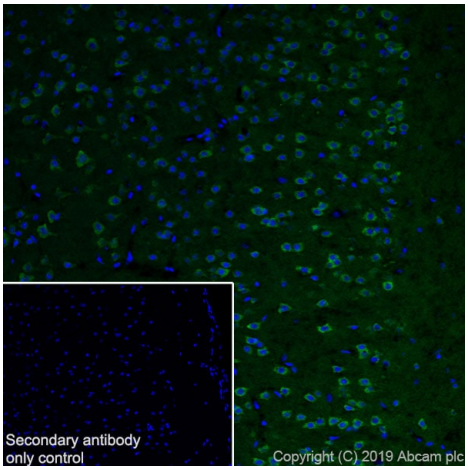
was observed when HTT knockout samples were exmined. Wild-type and HTT knockout samples were subjected to SDS-PAGE. Unpurified ab109115 and **ab18058** (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/10,000 dilution and 1/10,000 dilution respectively. Blots were developed with 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] (ab109115)

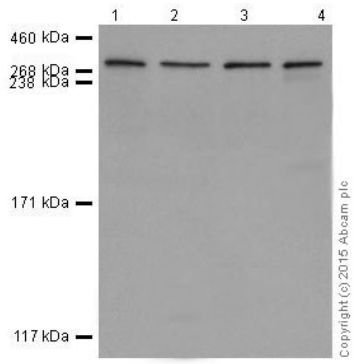
Immunohistochemical analysis of paraffin-embedded Human astrocytoma labeling Huntingtin with purified ab109115 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500. Counter stained with Hematoxylin. Nuclear staining on cancer cells of astrocytoma.

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



Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-Huntingtin antibody [EPR5526] (ab109115)

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.



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

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

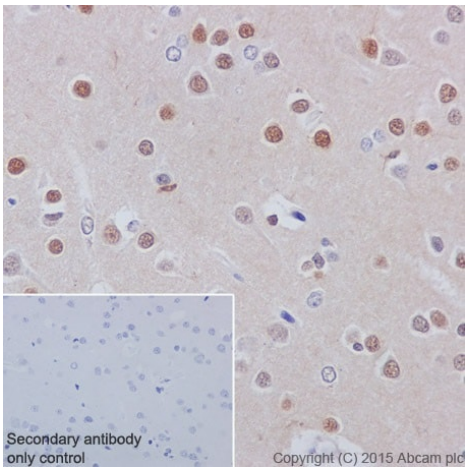
Lysates/proteins at 10 µg per lane.

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

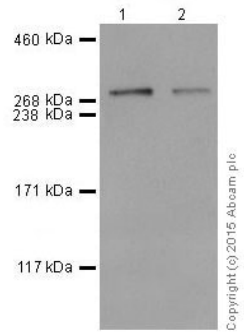
Blocking and Diluting buffer and concentration: 5% NFDM /TBST



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

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.



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

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

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

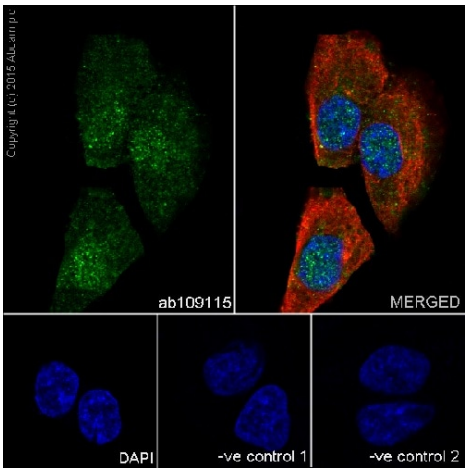
Lysates/proteins at 20 µg per lane.

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



Immunocytochemistry/ Immunofluorescence - Anti-Huntingtin antibody [EPR5526] (ab109115)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized

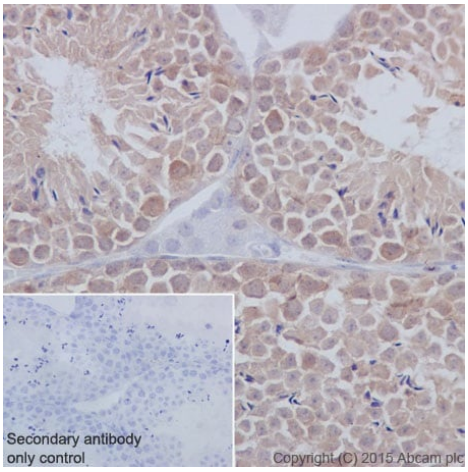
SH-SY5Y (Human neuroblastoma from bone marrow cells) cells labeling Huntingtin with purified ab109115 at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

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).

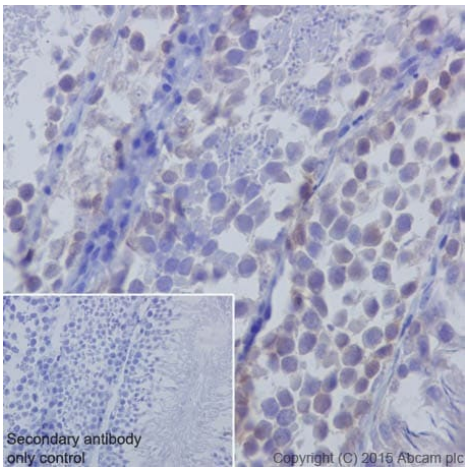
The negative controls are as follows:
1. **ab191472** at 1/1000 dilution followed by **ab150120** (Alexa Fluor® 594 Goat anti-Mouse secondary) at 1/1000 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor® 488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.



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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