Anti-Huntingtin Antibody [JB89-34]

ET7107-60

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Pig
Applications:	WB, IHC-P, FC, IHC-Fr, IF-Cell, IF-Tissue
Molecular Wt:	Predicted band size: 348 kDa
Clone number:	JB89-34

Description: Huntingtin (Htt) is the protein coded for by the HTT gene, also known as the IT15 ("interesting transcript 15") gene. Mutated HTT is the cause of Huntington's disease (HD), and has been investigated for this role and also for its involvement in long-term memory storage. It is variable in its structure, as the many polymorphisms of the gene can lead to variable numbers of glutamine residues present in the protein. In its wild-type (normal form), it contains 6-35 glutamine residues. However, in individuals affected by Huntington's disease (an autosomal dominant genetic disorder), it contains more than 36 glutamine residues (highest reported repeat length is about 250). Its commonly used name is derived from this disease; previously, the IT15 label was commonly used. Within cells, huntingtin may or may not be involved in signaling, transporting materials, binding proteins and other structures, and protecting against apoptosis, a form of programmed cell death. The huntingtin protein is required for normal development before birth. It is expressed in many tissues in the body, with the highest levels of expression seen in the brain.

Immunogen: Synthetic peptide within N-terminal Human Huntingtin .

Positive control: Neuro-2a cell lysate, PC-12 cell lysate, mouse brain tissue lysate, rat brain tissue lysate, human colon carcinoma tissue, human breast tissue, mouse epididymis tissue, rat brain tissue, SH-SY5Y.

Subcellular location: Cytoplasm. Nucleus.

Database links: SwissProt: P42858 Human | P42859 Mouse | P51111 Rat

Recommended Dilutions:

WB	1:5,000-1:10,000
IHC-P	1:200-1:1,000
FC	1:50-1:100
IHC-Fr	1:200
IF-Cell	1:100
IF-Tissue	1:100-1:200

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

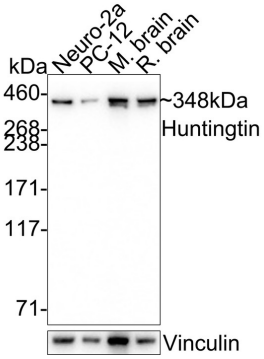

Fig1: Western blot analysis of Huntingtin on different lysates with Rabbit anti-Huntingtin antibody (ET7107-60) at 1/5,000 dilution.

Lane 1: Neuro-2a cell lysate (15 µg/Lane)
Lane 2: PC-12 cell lysate (15 µg/Lane)
Lane 3: Mouse brain tissue lysate (20 µg/Lane)
Lane 4: Rat brain tissue lysate (20 µg/Lane)

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

Exposure time: 2 minutes 17 seconds;

3-8% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET7107-60) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

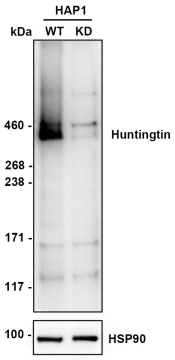

Fig2: Western blot analysis of Huntingtin on different lysates with Rabbit anti-Huntingtin antibody (ET7107-60) at 1/10,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-Huntingtin KD cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 100 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET7107-60) at 1/10,000 dilution was used in K1803 at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

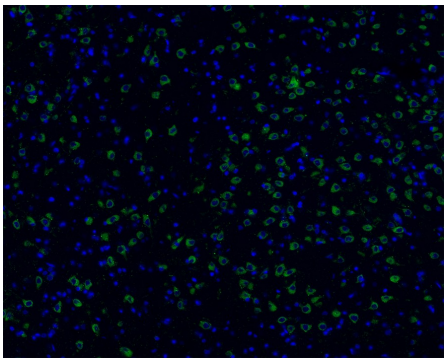

Fig3: Application: IHC-Fr

Species: Mouse

Site: Cerebral cortex

Sample: Frozen section

Antibody concentration: 1/200

Antigen retrieval: Not required

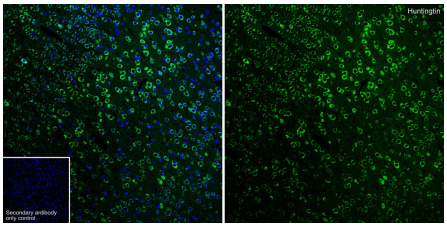

Fig4: Application: IF-tissue

Species: Mouse

Site: Cerebral cortex

Sample: Paraffin-embedded section

Antibody concentration: 1/200

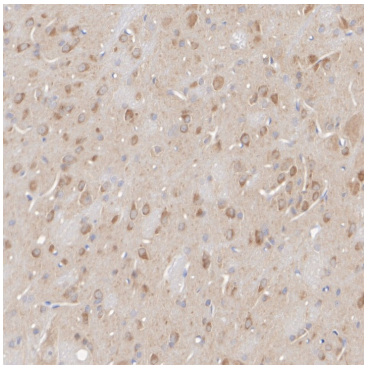

Fig5: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-Huntingtin antibody (ET7107-60) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7107-60) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

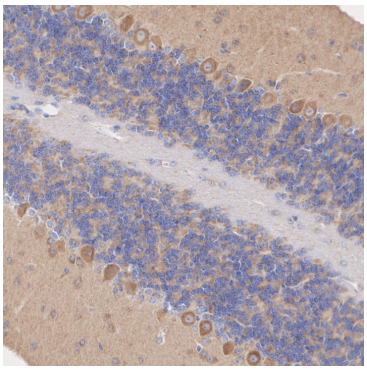

Fig6: Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue with Rabbit anti-Huntingtin antibody (ET7107-60) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7107-60) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

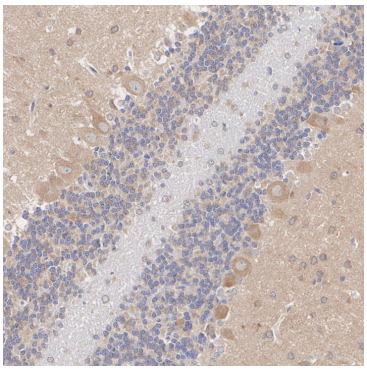

Fig7: Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue with Rabbit anti-Huntingtin antibody (ET7107-60) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7107-60) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

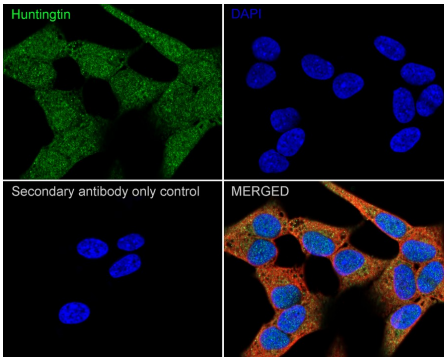

Fig8: Immunocytochemistry analysis of SH-SY5Y cells labeling Huntingtin with Rabbit anti-Huntingtin antibody (ET7107-60) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Huntingtin antibody (ET7107-60) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

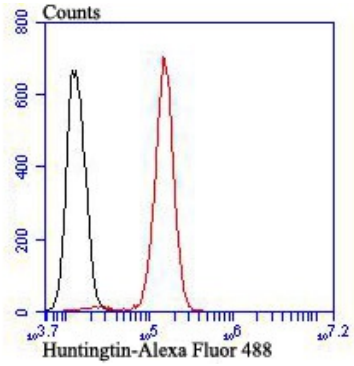

Fig9: Flow cytometric analysis of Huntingtin was done on SH-SY5Y cells. The cells were fixed, permeabilized and stained with the primary antibody (ET7107-60, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG Secondary antibody at 1/1,000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Cornett J et al. Polyglutamine expansion of huntingtin impairs its nuclear export. Nat Genet 37:198-204 (2005).
2. Sayer J A et al. Interaction of the nuclear matrix protein NAKAP with HypA and huntingtin: implications for nuclear toxicity in Huntington's disease pathogenesis. NeuroMolecular Med 7:297-310 (2005).