Anti-Parkin Antibody [PSH08-11] - BSA and Azide free

HA751206

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey, Cynomolgus monkey, Pig
Applications:	WB, IHC-P, IF-Tissue, IF-Cell, FC, IP, IHC-Fr
Molecular Wt:	Predicted band size: 52 kDa
Clone number:	PSH08-11

Description: Parkin is a 465-amino acid residue E3 ubiquitin ligase, a protein that in humans and mice is encoded by the PARK2 gene. Parkin plays a critical role in ubiquitination – the process whereby molecules are covalently labelled with ubiquitin (Ub) and directed towards degradation in proteasomes or lysosomes. Ubiquitination involves the sequential action of three enzymes. First, an E1 ubiquitin-activating enzyme binds to inactive Ub in eukaryotic cells via a thioester bond and mobilises it in an ATP-dependent process. Ub is then transferred to an E2 ubiquitin-conjugating enzyme before being conjugated to the target protein via an E3 ubiquitin ligase. There exists a multitude of E3 ligases, which differ in structure and substrate specificity to allow selective targeting of proteins to intracellular degradation.

Immunogen: Recombinant protein within human Parkin aa 1-200.

Positive control: SH-SY5Y cell lysate, 293T cell lysate, COS-1 cell lysate, Neuro-2a cell lysate, C6 cell lysate, Mouse brain tissue lysate, Rat brain tissue lysate, mouse brain tissue, rat brain tissue, SH-SY5Y, Neuro-2a, C6.

Subcellular location: Mitochondrion, mitochondrion outer membrane, endoplasmic reticulum, cytosol, Nucleus, neuron projection, postsynaptic density, presynapse.

Database links: SwissProt: O60260 Human | Q9WVS6 Mouse | Q9JK66 Rat

Recommended Dilutions:

WB	1:2,000-1:10,000
IHC-P	1:1,000
IF-Tissue	1:200
IF-Cell	1:50-1:100
FC	1:1,000
IP	IP: 1-2μg/sample
IHC-Fr	1:500

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

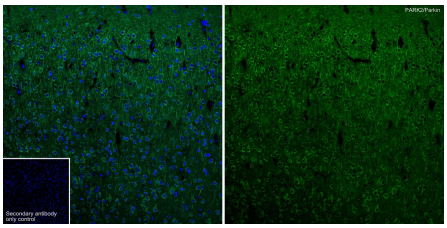

Fig1: Application: IHC-Fr

Species: Mouse

Site: Cerebral cortex

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven.

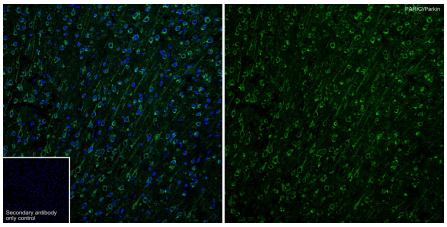

Fig2: Application: IHC-Fr

Species: Rat

Site: Cerebral cortex

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven.

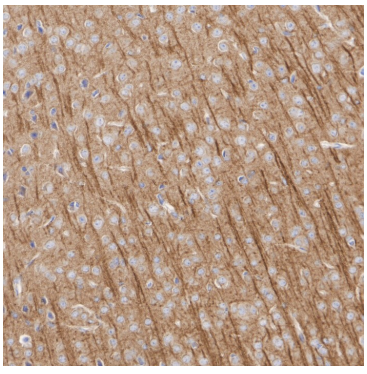

Fig3: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-Parkin antibody (HA751206) at 1/1,000 dilution.

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 (HA751206) 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.

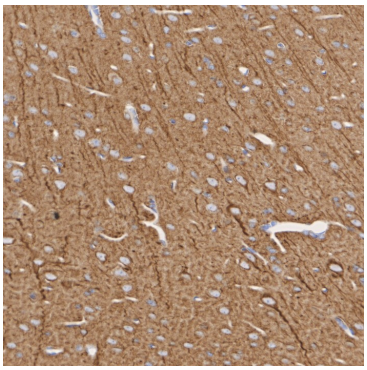

Fig4: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-Parkin antibody (HA751206) at 1/1,000 dilution.

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 (HA751206) 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.

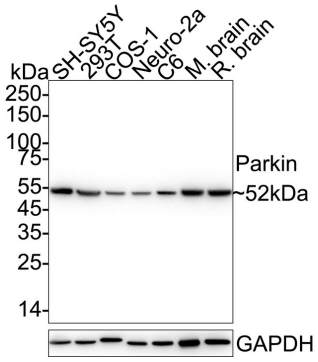

Fig5: Western blot analysis of Parkin on different lysates with Rabbit anti-Parkin antibody (HA751206) at 1/2,000 dilution.

Lane 1: SH-SY5Y cell lysate (20 µg/Lane)
Lane 2: 293T cell lysate (20 µg/Lane)
Lane 3: COS-1 cell lysate (20 µg/Lane)
Lane 4: Neuro-2a cell lysate (20 µg/Lane)
Lane 5: C6 cell lysate (20 µg/Lane)
Lane 6: Mouse brain tissue lysate (30 µg/Lane)
Lane 7: Rat brain tissue lysate (30 µg/Lane)

Predicted band size: 52 kDa
Observed band size: 52 kDa

Exposure time: 6 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 (HA751206) at 1/2,000 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.

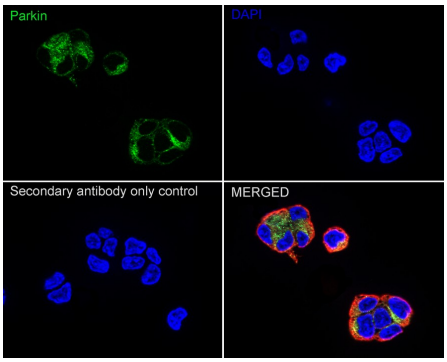

Fig6: Immunocytochemistry analysis of SH-SY5Y cells labeling Parkin with Rabbit anti-Parkin antibody (HA751206) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-Parkin antibody (HA751206) 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 (HA601187, 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.

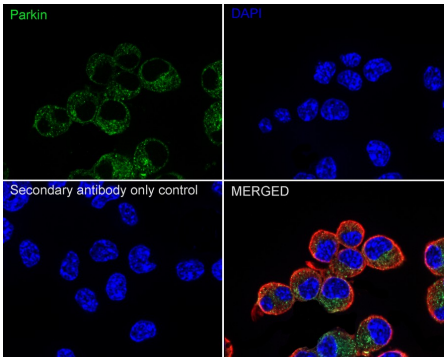

Fig7: Immunocytochemistry analysis of Neuro-2a cells labeling Parkin with Rabbit anti-Parkin antibody (HA751206) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-Parkin antibody (HA751206) at 1/50 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 (HA601187, 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.

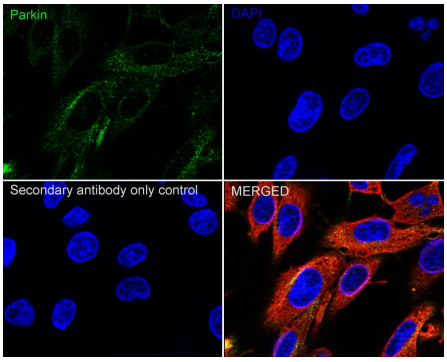

Fig8: Immunocytochemistry analysis of C6 cells labeling Parkin with Rabbit anti-Parkin antibody (HA751206) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-Parkin antibody (HA751206) at 1/50 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 (HA601187, 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.

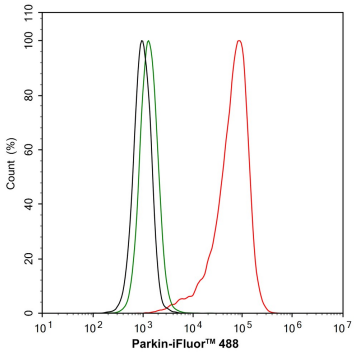

Fig9: Flow cytometric analysis of SH-SY5Y cells labeling Parkin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751206, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

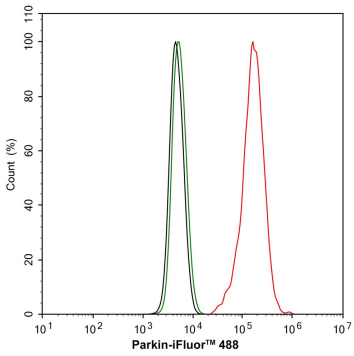

Fig10: Flow cytometric analysis of Neuro-2a cells labeling Parkin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751206, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

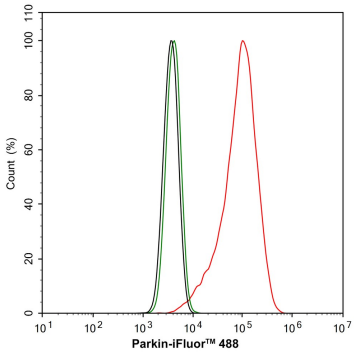

Fig11: Flow cytometric analysis of C6 cells labeling Parkin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751206, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

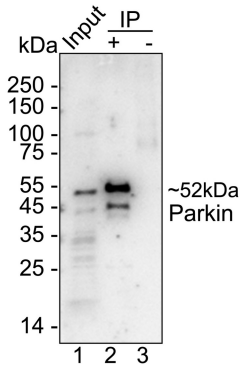

Fig12: Parkin was immunoprecipitated from 0.2 mg SH-SY5Y cell lysate with HA751206 at 2 μg/25 μl agarose. Western blot was performed from the immunoprecipitate using Mouse anti-Parkin antibody at 1/1,000 dilution. Anti-Mouse IgG for IP Nano-secondary antibody (NBI02H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: SH-SY5Y cell lysate (input)
Lane 2: HA751206 IP in SH-SY5Y cell lysate
Lane 3: Mouse IgG instead of HA751206 in SH-SY5Y cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 2 minutes; ECL: K1801

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

Background References

1. Quinn PMJ et al. PINK1/PARKIN signalling in neurodegeneration and neuroinflammation. Acta Neuropathol Commun. 2020 Nov
2. Li J et al. PINK1/Parkin-mediated mitophagy in neurodegenerative diseases. Ageing Res Rev. 2023 Feb