

Anti-USP5 Antibody [JE77-42]

HA722371



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 96 kDa
Clone number:	JE77-42

Description: Cleaves linear and branched multiubiquitin polymers with a marked preference for branched polymers. Involved in unanchored 'Lys-48'-linked polyubiquitin disassembly. Binds linear and 'Lys-63'-linked polyubiquitin with a lower affinity. Knock-down of USP5 causes the accumulation of p53/TP53 and an increase in p53/TP53 transcriptional activity because the unanchored polyubiquitin that accumulates is able to compete with ubiquitinated p53/TP53 but not with MDM2 for proteasomal recognition.

Immunogen: Recombinant protein within Human USP5 aa 759-858 / 858.

Positive control: HeLa cell lysate, A549 cell lysate, K-562 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, mouse brain tissue lysate, rat brain tissue lysate, HeLa, NIH/3T3, PC-12, human brain tissue, human kidney tissue.

Subcellular location: Cytosol, lysosome, nucleus.

Database links: SwissProt: P45974 Human | P56399 Mouse
Entrez Gene: 297593 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:50
IHC-P	1:200
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

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Orders:0086-571-88062880

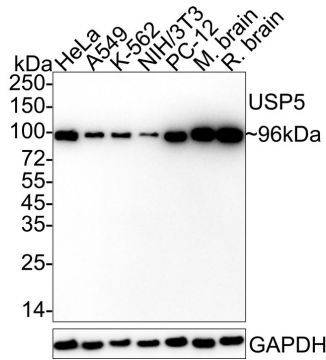
Technical:0086-571-89986345

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Images

Fig1: Western blot analysis of USP5 on different lysates with Rabbit anti-USP5 antibody (HA722371) at 1/1,000 dilution.



Lane 1: HeLa cell lysate
 Lane 2: A549 cell lysate
 Lane 3: K-562 cell lysate
 Lane 4: NIH/3T3 cell lysate
 Lane 5: PC-12 cell lysate
 Lane 6: Mouse brain tissue lysate
 Lane 7: Rat brain tissue lysate

Lysates/proteins at 20 µg/Lane.

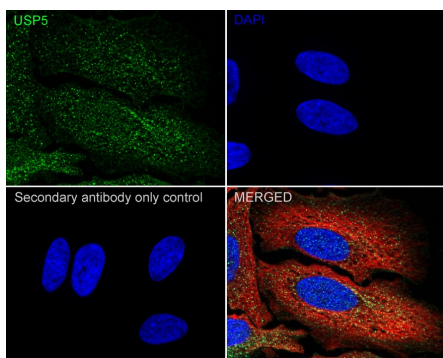
Predicted band size: 96 kDa
 Observed band size: 96 kDa

Exposure time: 3 minutes; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (HA722371) at 1/1,000 dilution was used in 5% NFDN/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Immunocytochemistry analysis of HeLa cells labeling USP5 with Rabbit anti-USP5 antibody (HA722371) at 1/50 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-USP5 antibody (HA722371) at 1/50 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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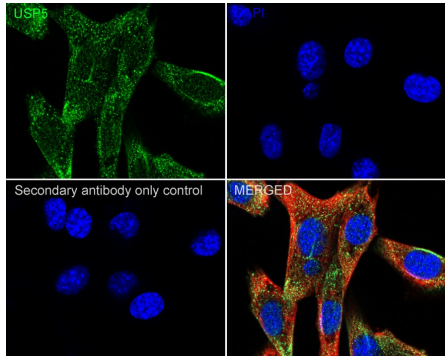
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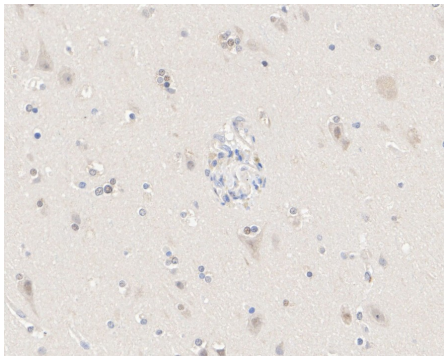
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Fig3: Immunocytochemistry analysis of NIH/3T3 cells labeling USP5 with Rabbit anti-USP5 antibody (HA722371) at 1/50 dilution.



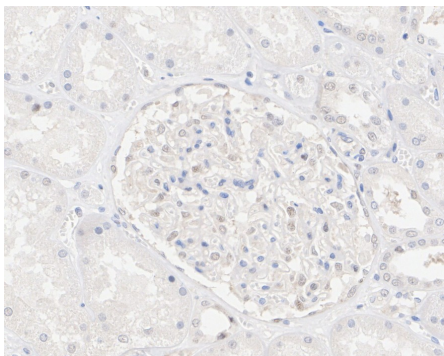
Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-USP5 antibody (HA722371) at 1/50 dilution in 1% BSA in PBST overnight at 4 °C. 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 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig4: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-USP5 antibody (HA722371) at 1/200 dilution.



The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA722371) 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.

Fig5: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-USP5 antibody (HA722371) at 1/200 dilution.



The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA722371) 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.

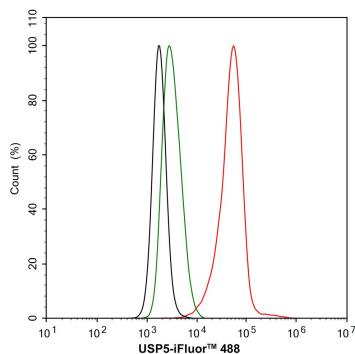


Fig6: Flow cytometric analysis of HeLa cells labeling USP5.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722371, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C 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°C. 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. Xiao X et al. ERK and USP5 govern PD-1 homeostasis via deubiquitination to modulate tumor immunotherapy. *Nat Commun.* 2023 May
2. Cai B et al. USP5 attenuates NLRP3 inflammasome activation by promoting autophagic degradation of NLRP3. *Autophagy.* 2022 May

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