Anti-YTHDF1 Antibody [PSH0-23]

HA721302

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, FC
Molecular Wt:	Predicted band size: 61 kDa
Clone number:	PSH0-23

Description: Specifically recognizes and binds N6-methyladenosine (m6A)-containing mRNAs, and regulates their stability. M6A is a modification present at internal sites of mRNAs and some non-coding RNAs and plays a role in mRNA stability and processing. Acts as a regulator of mRNA stability by promoting degradation of m6A-containing mRNAs via interaction with the CCR4-NOT complex. The YTHDF paralogs (YTHDF1, YTHDF2 and YTHDF3) shares m6A-containing mRNAs targets and act redundantly to mediate mRNA degradation and cellular differentiation. Required to facilitate learning and memory formation in the hippocampus by binding to m6A-containing neuronal mRNAs. Acts as a regulator of axon guidance by binding to m6A-containing ROBO3 transcripts. Acts as a negative regulator of antigen cross-presentation in myeloid dendritic cells. In the context of tumorigenesis, negative regulation of antigen cross-presentation limits the anti-tumor response by reducing efficiency of tumor-antigen cross-presentation. Promotes formation of phase-separated membraneless compartments, such as P-bodies or stress granules, by undergoing liquid-liquid phase separation upon binding to mRNAs containing multiple m6A-modified residues: polymethylated mRNAs act as a multivalent scaffold for the binding of YTHDF proteins, juxtaposing their disordered regions and thereby leading to phase separation. The resulting mRNA-YTHDF complexes then partition into different endogenous phase-separated membraneless compartments, such as P-bodies, stress granules or neuronal RNA granules.

Immunogen: Recombinant protein within human YTHDF1 aa 300-559.

Positive control: HepG2 cell lysate, Hela cell lysate, 293T cell lysate, MCF-7 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, HCT116 cell lysate, mouse testis liver tissue lysate, rat testis tissue lysate, mouse brain tissue lysate, rat brain tissue lysate, human brain tissue, mouse brain tissue, mouse hippocampus tissue, rat brain tissue, 293, PC-12.

Subcellular location: Cytoplasm, P-body.

Database links: SwissProt: Q9BYJ9 Human | P59326 Mouse
Entrez Gene: 296467 Rat

Recommended Dilutions:

WB	1:1,000
IHC-P	1:1,000
FC	1ug/mL

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Images

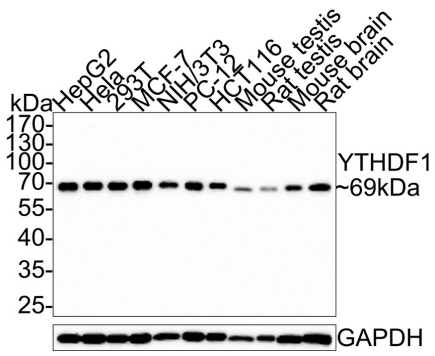

Fig1: Western blot analysis of YTHDF1 on different lysates with Rabbit anti-YTHDF1 antibody (HA721302) at 1/1,000 dilution.

Lane 1: HepG2 cell lysate (10 µg/Lane)
Lane 2: Hela cell lysate (10 µg/Lane)
Lane 3: 293T cell lysate (10 µg/Lane)
Lane 4: MCF-7 cell lysate (10 µg/Lane)
Lane 5: NIH/3T3 cell lysate (10 µg/Lane)
Lane 6: PC-12 cell lysate (10 µg/Lane)
Lane 7: HCT116 cell lysate (10 µg/Lane)
Lane 8: Mouse testis liver tissue lysate (20 µg/Lane)
Lane 9: Rat testis tissue lysate (20 µg/Lane)
Lane 10: Mouse brain tissue lysate (20 µg/Lane)
Lane 11: Rat brain tissue lysate (20 µg/Lane)

Predicted band size: 61 kDa
Observed band size: 69 kDa

Exposure time: 5 minutes;

10% 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 (HA721302) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

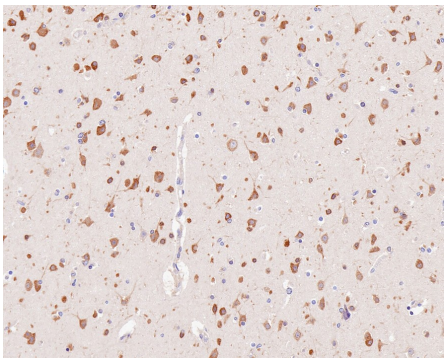

Fig2: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-YTHDF1 antibody (HA721302) at 1/1,000 dilution.

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 (HA721302) 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.

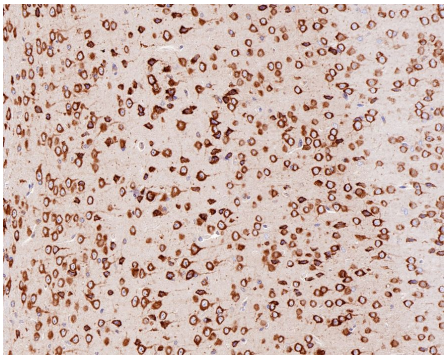

Fig3: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-YTHDF1 antibody (HA721302) at 1/1,000 dilution.

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 (HA721302) 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.

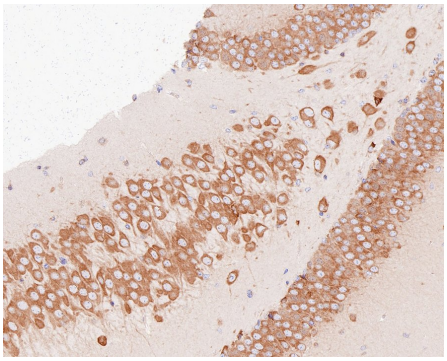

Fig4: Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue with Rabbit anti-YTHDF1 antibody (HA721302) at 1/1,000 dilution.

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 (HA721302) 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.

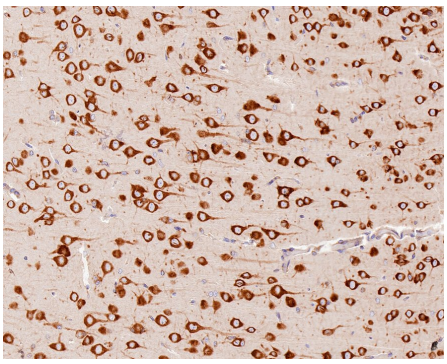

Fig5: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-YTHDF1 antibody (HA721302) at 1/1,000 dilution.

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 (HA721302) 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.

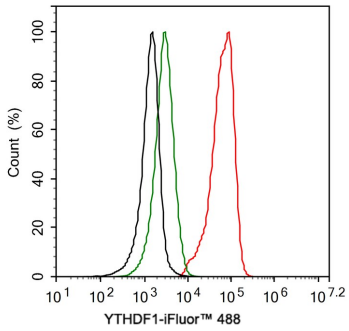

Fig6: Flow cytometric analysis of 293 cells labeling YTHDF1.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721302, 1ug/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).

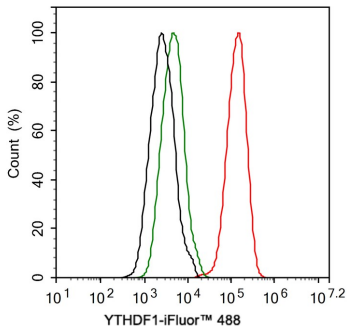

Fig7: Flow cytometric analysis of PC-12 cells labeling YTHDF1.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721302, 1ug/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).

Background References

1. Gao Y., Pei G., Li D., Li R., Shao Y., Zhang Q.C., Li P. Multivalent m6A motifs promote phase separation of YTHDF proteins. Cell Res. 29:767-769(2019) [PubMed] [Europe PMC]
2. Zaccara S., Jaffrey S.R. A unified model for the function of YTHDF proteins in regulating m6A-modified mRNA. Cell 181:1582-1595(2020) [PubMed] [Europe PMC]