Anti-YTHDF2 Antibody [PSH06-17]

HA722514

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IHC-P, FC
Molecular Wt:	Predicted band size: 62 kDa
Clone number:	PSH06-17

Description: Specifically recognizes and binds N6-methyladenosine (m6A)-containing RNAs, 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 and ribonuclease P/MRP complexes, depending on the context. The YTHDF paralogs (YTHDF1, YTHDF2 and YTHDF3) share m6A-containing mRNAs targets and act redundantly to mediate mRNA degradation and cellular differentiation. M6A-containing mRNAs containing a binding site for RIDA/HRSP12 (5'-GGUUC-3') are preferentially degraded by endoribonucleolytic cleavage: cooperative binding of RIDA/HRSP12 and YTHDF2 to transcripts leads to recruitment of the ribonuclease P/MRP complex. Other m6A-containing mRNAs undergo deadenylation via direct interaction between YTHDF2 and CNOT1, leading to recruitment of the CCR4-NOT and subsequent deadenylation of m6A-containing mRNAs. Required maternally to regulate oocyte maturation: probably acts by binding to m6A-containing mRNAs, thereby regulating maternal transcript dosage during oocyte maturation, which is essential for the competence of oocytes to sustain early zygotic development. Also required during spermatogenesis: regulates spermagonial adhesion by promoting degradation of m6A-containing transcripts coding for matrix metallopeptidases. Also involved in hematopoietic stem cells specification by binding to m6A-containing mRNAs, leading to promote their degradation. Also acts as a regulator of neural development by promoting m6A-dependent degradation of neural development-related mRNA targets.

Immunogen: Synthetic peptide within Human YTHDF2 aa 519-579.

Positive control: HEK-293 cell lysate, HeLa cell lysate, Jurkat cell lysate, HCT 116 cell lysate, MDA-MB-231 cell lysate, COS-1 cell lysate, human testis tissue, mouse brain tissue, mouse testis tissue, rat brain tissue, rat testis tissue, HCT 116.

Subcellular location: Cytoplasm. Nucleus.

Database links: SwissProt: Q9Y5A9 Human | Q91YT7 Mouse
Entrez Gene: 313053 Rat

Recommended Dilutions:

WB	1:2,000
IHC-P	1:500
FC	1:1,000

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

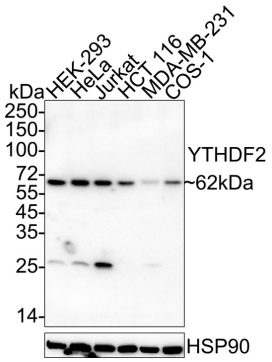

Fig1: Western blot analysis of YTHDF2 on different lysates with Rabbit anti-YTHDF2 antibody (HA722514) at 1/2,000 dilution.

Lane 1: HEK-293 cell lysate
Lane 2: HeLa cell lysate
Lane 3: Jurkat cell lysate
Lane 4: HCT 116 cell lysate
Lane 5: MDA-MB-231 cell lysate
Lane 6: COS-1 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 62 kDa
Observed band size: 62 kDa

Exposure time: 3 minutes; 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 (HA722514) 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.

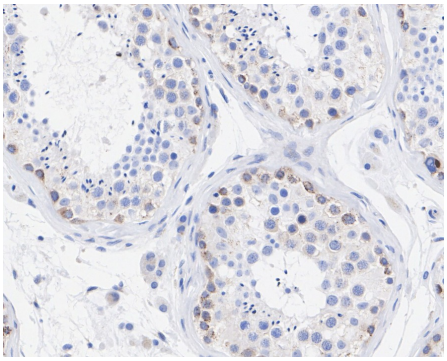

Fig2: Immunohistochemical analysis of paraffin-embedded human testis tissue with Rabbit anti-YTHDF2 antibody (HA722514) at 1/500 dilution.

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 (HA722514) at 1/500 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.

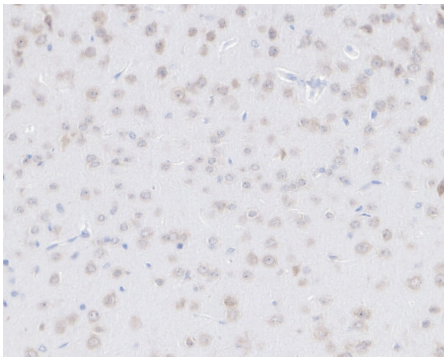

Fig3: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-YTHDF2 antibody (HA722514) at 1/500 dilution.

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 (HA722514) at 1/500 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.

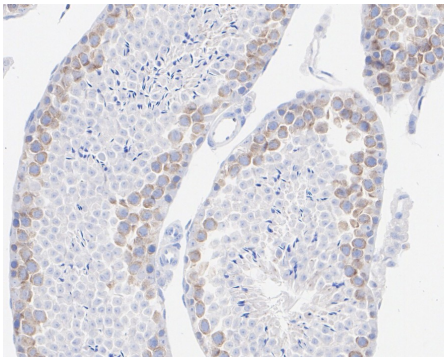

Fig4: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Rabbit anti-YTHDF2 antibody (HA722514) at 1/500 dilution.

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 (HA722514) at 1/500 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.

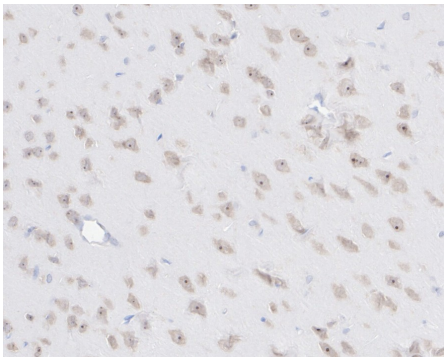

Fig5: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-YTHDF2 antibody (HA722514) at 1/500 dilution.

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 (HA722514) at 1/500 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.

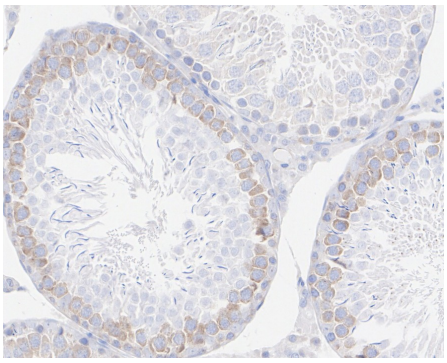

Fig6: Immunohistochemical analysis of paraffin-embedded rat testis tissue with Rabbit anti-YTHDF2 antibody (HA722514) at 1/500 dilution.

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 (HA722514) at 1/500 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.

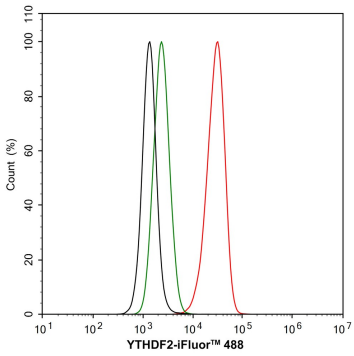

Fig7: Flow cytometric analysis of HCT 116 cells labeling YTHDF2.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722514, 1/1,000) (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).

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

Background References

1. Wang X., Lu Z., Gomez A., Hon G.C., Yue Y., Han D., Fu Y., Parisien M., Dai Q., Jia G., Ren B., Pan T., He C. N-methyladenosine-dependent regulation of messenger RNA stability. Nature 505:117-120(2014)
2. Du H., Zhao Y., He J., Zhang Y., Xi H., Liu M., Ma J., Wu L. YTHDF2 destabilizes m(6)A-containing RNA through direct recruitment of the CCR4-NOT deadenylase complex. Nat. Commun. 7:12626-12626(2016)