Anti-METTL14 Antibody [PSH07-85]

HA722910

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IF-Cell, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 52 kDa
Clone number:	PSH07-85

Description: The METTL3-METTL14 heterodimer forms a N6-methyltransferase complex that methylates adenosine residues at the N(6) position of some mRNAs and regulates the circadian clock, differentiation of embryonic stem cells and cortical neurogenesis. In the heterodimer formed with METTL3, METTL14 constitutes the RNA-binding scaffold that recognizes the substrate rather than the catalytic core. N6-methyladenosine (m6A), which takes place at the 5'-[AG]GAC-3' consensus sites of some mRNAs, plays a role in mRNA stability and processing. M6A acts as a key regulator of mRNA stability by promoting mRNA destabilization and degradation (By similarity). In embryonic stem cells (ESCs), m6A methylation of mRNAs encoding key naive pluripotency-promoting transcripts results in transcript destabilization (By similarity). M6A regulates spermatogonial differentiation and meiosis and is essential for male fertility and spermatogenesis (By similarity). M6A also regulates cortical neurogenesis: m6A methylation of transcripts related to transcription factors, neural stem cells, the cell cycle and neuronal differentiation during brain development promotes their destabilization and decay, promoting differentiation of radial glial cells (By similarity).

Immunogen: Recombinant protein within human METTL14 aa 1-150.

Positive control: A431 cell lysate, Raji cell lysate, PC-12 cell lysate, COS-1 cell lysate, Mouse testis tissue lysate, Rat testis tissue lysate, A431, human kidney tissue, human lung tissue, mouse testis tissue, rat kidney tissue, rat lung tissue, rat testis tissue.

Subcellular location: Nucleus.

Database links: SwissProt: Q9HCE5 Human | Q3UIK4 Mouse
Entrez Gene: 295428 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:100
IHC-P	1:200-1:1,000
FC	1:1,000
IP	1-2μg/sample

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

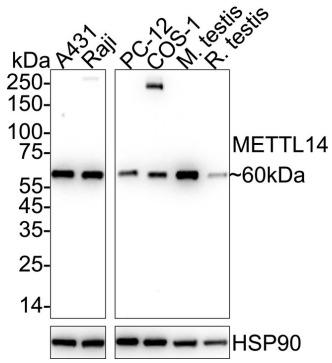

Fig1: Western blot analysis of METTL14 on different lysates with Rabbit anti-METTL14 antibody (HA722910) at 1/5,000 dilution.

Lane 1: A431 cell lysate (20 µg/Lane)
Lane 2: Raji cell lysate (20 µg/Lane)
Lane 3: PC-12 cell lysate (20 µg/Lane)
Lane 4: COS-1 cell lysate (20 µg/Lane)
Lane 5: Mouse testis tissue lysate (40 µg/Lane)
Lane 6: Rat testis tissue lysate (40 µg/Lane)

Predicted band size: 52 kDa
Observed band size: 60 kDa

Exposure time: 30 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 (HA722910) 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.

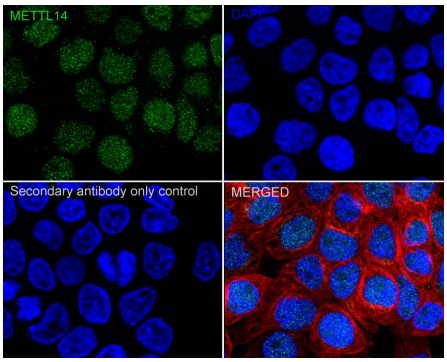

Fig2: Immunocytochemistry analysis of A431 cells labeling METTL14 with Rabbit anti-METTL14 antibody (HA722910) at 1/100 dilution.

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-METTL14 antibody (HA722910) 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.

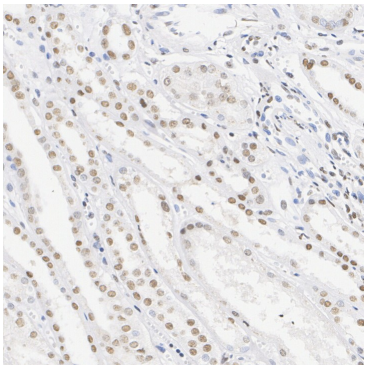

Fig3: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-METTL14 antibody (HA722910) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA722910) 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.

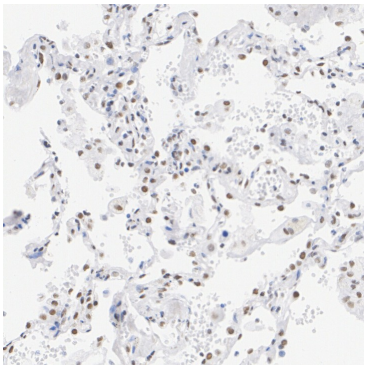

Fig4: Immunohistochemical analysis of paraffin-embedded human lung tissue with Rabbit anti-METTL14 antibody (HA722910) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA722910) 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.

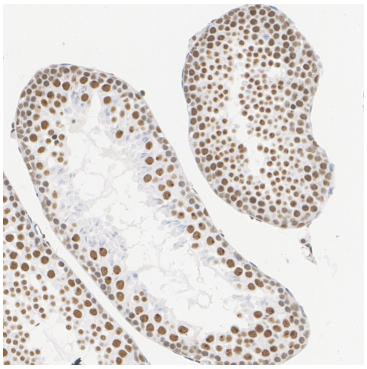

Fig5: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Rabbit anti-METTL14 antibody (HA722910) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA722910) 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.

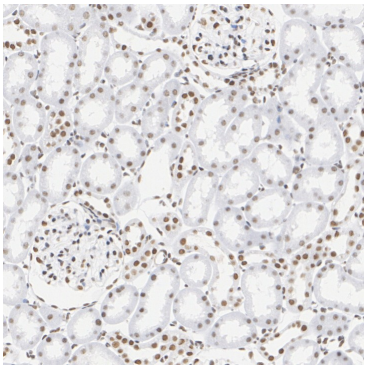

Fig6: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-METTL14 antibody (HA722910) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA722910) 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.

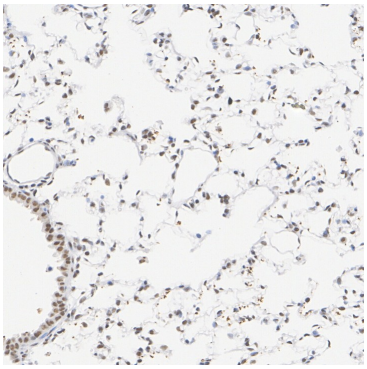

Fig7: Immunohistochemical analysis of paraffin-embedded rat lung tissue with Rabbit anti-METTL14 antibody (HA722910) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA722910) 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.

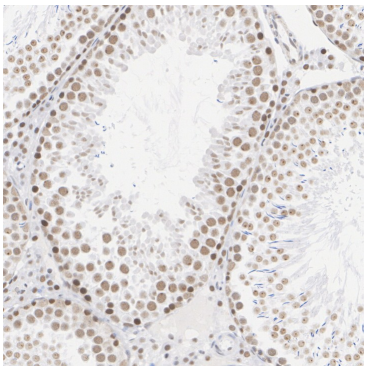

Fig8: Immunohistochemical analysis of paraffin-embedded rat testis tissue with Rabbit anti-METTL14 antibody (HA722910) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA722910) 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.

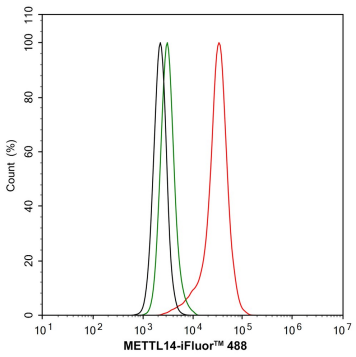

Fig9: Flow cytometric analysis of A431 cells labeling METTL14.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722910, 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).

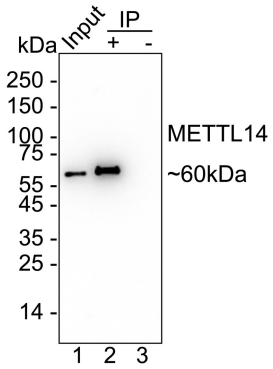

Fig10: METTL14 was immunoprecipitated from 0.2 mg A431 cell lysate with HA722910 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA722910 at 1/5,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: A431 cell lysate (input)
Lane 2: HA722910 IP in A431 cell lysate
Lane 3: Rabbit IgG instead of HA722910 in A431 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 1 minute; ECL: K1801

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

Background References

1. Guan Q et al. Functions, mechanisms, and therapeutic implications of METTL14 in human cancer. J Hematol Oncol. 2022 Feb
2. Meng L et al. METTL14 suppresses pyroptosis and diabetic cardiomyopathy by downregulating TINCR lncRNA. Cell Death Dis. 2022 Jan