m6A Essentials Antibody Sampler Kit

HAK21121

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
m6A [HA721152]	20μl	Dot Blot,ELISA	Si	
METTL3 [HA720002]	20μl	WB,IHC-P,IF-Cell	H,M,R	64 kDa
METTL14 [HA722910]	20μl	WB,IF-Cell,IHC-P,FC,IP	H,M,R,Mk	52 kDa
WTAP [HA500525]	20μl	WB,IHC-P,IF-Cell	H,M,R	44 kDa
FTO [ET1705-89]	20μl	WB,IHC-P,IF-Cell	H,M,R	58 kDa
ALKBH5 [HA721628]	20μl	WB,IHC-P	H,M,R	44 kDa
YTHDF1 [HA721302]	20μl	WB,IHC-P,FC	H,M,R	61 kDa
YTHDF2 [HA722514]	20μl	WB,IHC-P,FC	H,M,R,Mk	62 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100μl	WB,ELISA,IHC-P	Rab	

Description: The m6A Essentials Antibody Kit provides a cost-effective tool for studying modified m6A and its regulators. It is ideal for researchers starting new projects, screening for multiple potential targets, or those who simply need a smaller volume of antibody.

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

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

Background m6A (n6-methyladenosine) is the most abundant modification in mammalian mRNA. This modification is initiated by m6A methyltransferases (Writers), such as METTL3, METTL14, and WTAP. The m6A modification can be reversed by demethylases (Erasers) such as FTO and ALKBH5. The stability of m6A-modified mRNA is regulated by the YTHDF protein (Readers), which recognizes m6A and reduces the stability of the target transcript. m6A and its regulatory proteins play a key role in the initiation and progression of cancer.

Database links: UniProt ID: NoneQ86U44, Q8C3P7, 361035, Q9HCE5, Q3UIK4, 295428, Q15007, Q9ER69, 499020, Q9C0B1, Q8BGW1, Q2A121, Q6P6C2, Q3TSG4, D3ZKD3, Q9BYJ9, P59326, 296467, Q9Y5A9, Q91YT7, 313053

Images

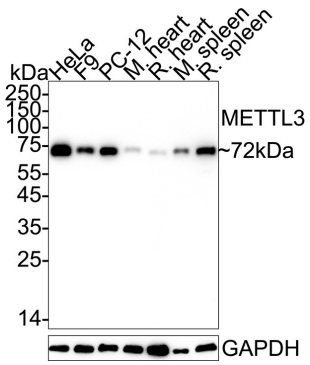

Fig1: Western blot analysis of METTL3 on different lysates with Rabbit anti-METTL3 antibody (HA720002) at 1/1,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: F9 cell lysate (20 µg/Lane)
Lane 3: PC-12 cell lysate (20 µg/Lane)
Lane 4: Mouse heart tissue lysate (40 µg/Lane)
Lane 5: Rat heart tissue lysate (40 µg/Lane)
Lane 6: Mouse spleen tissue lysate (40 µg/Lane)
Lane 7: Rat spleen tissue lysate (40 µg/Lane)

Predicted band size: 64 kDa
Observed band size: 72 kDa

Exposure time: 25 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 (HA720002) at 1/1,000 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.

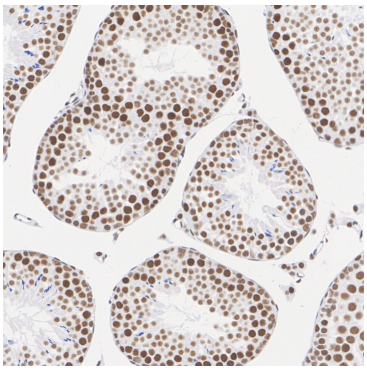

Fig2: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Rabbit anti-METTL3 antibody (HA720002) at 1/1,000 dilution.

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 (HA720002) 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.

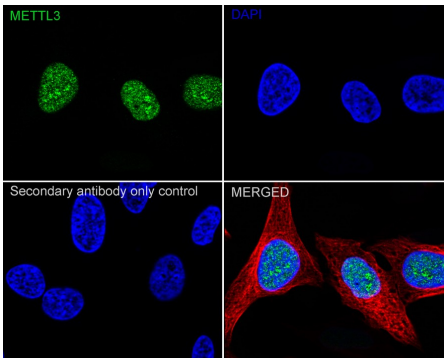

Fig3: Immunocytochemistry analysis of HeLa cells labeling METTL3 with Rabbit anti-METTL3 antibody (HA720002) at 1/100 dilution.

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-METTL3 antibody (HA720002) 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.

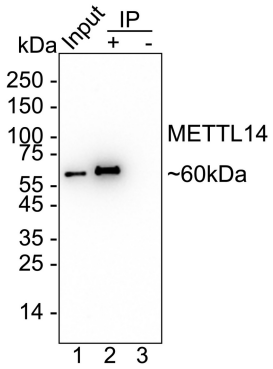

Fig4: METTL14 was immunoprecipitated from 0.2 mg A431 cell lysate with HA722910 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA722910 at 1/5,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

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

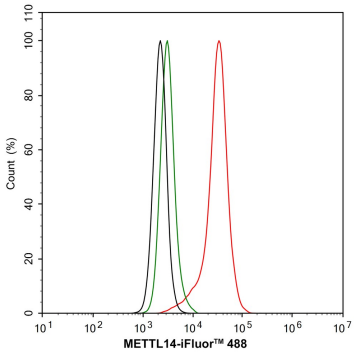

Fig5: Flow cytometric analysis of A431 cells labeling METTL14.

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).

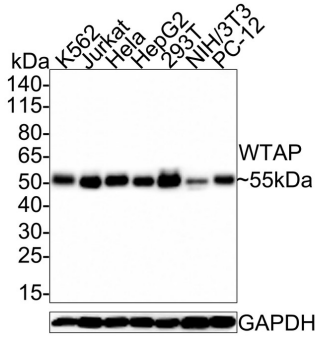

Fig6: Western blot analysis of WTAP on different lysates with Rabbit anti-WTAP antibody (HA500525) at 1/1,000 dilution.

Lane 1: K562 cell lysate (12.5 µg/Lane)
Lane 2: Jurkat cell lysate (10 µg/Lane)
Lane 3: Hela cell lysate (10 µg/Lane)
Lane 4: HepG2 cell lysate (10 µg/Lane)
Lane 5: 293T cell lysate (10 µg/Lane)
Lane 6: NIH/3T3 cell lysate (10 µg/Lane)
Lane 7: PC-12 cell lysate (10 µg/Lane)

Predicted band size: 44 kDa
Observed band size: 55 kDa

Exposure time: 30 seconds;

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 (HA500525) 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 1:200,000 dilution was used for 1 hour at room temperature.

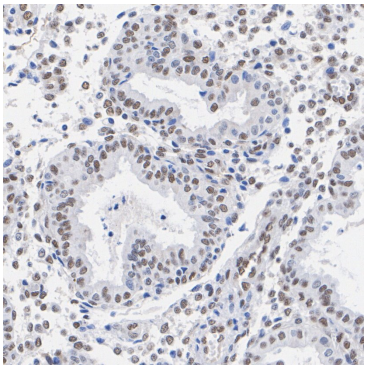

Fig7: Immunohistochemical analysis of paraffin-embedded human uterus tissue with Rabbit anti-FTO antibody (ET1705-89) at 1/1,000 dilution.

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 (ET1705-89) 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.

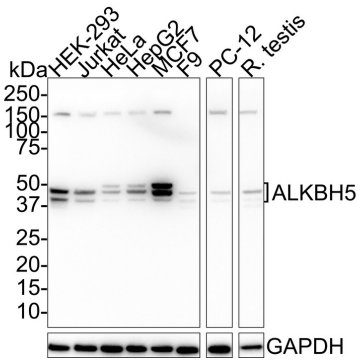

Fig8: Western blot analysis of ALKBH5 on different lysates with Rabbit anti-ALKBH5 antibody (HA721628) at 1/1,000 dilution.

Lane 1: HEK-293 cell lysate (20 µg/Lane)
Lane 2: Jurkat cell lysate (20 µg/Lane)
Lane 3: HeLa cell lysate (20 µg/Lane)
Lane 4: HepG2 cell lysate (20 µg/Lane)
Lane 5: MCF7 cell lysate (20 µg/Lane)
Lane 6: F9 cell lysate (20 µg/Lane)
Lane 7: PC-12 cell lysate (20 µg/Lane)
Lane 8: Rat testis tissue lysate (40 µg/Lane)

Predicted band size: 44 kDa
Observed band size: 35~44 kDa

Exposure time: 30 seconds;

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 (HA721628) 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 1:100,000 dilution was used for 1 hour at room temperature.

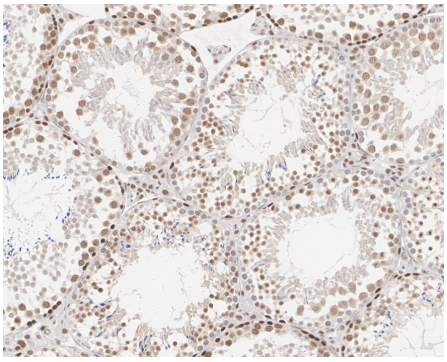

Fig9: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Rabbit anti-ALKBH5 antibody (HA721628) at 1/2,000 dilution.

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 (HA721628) at 1/2,000 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.

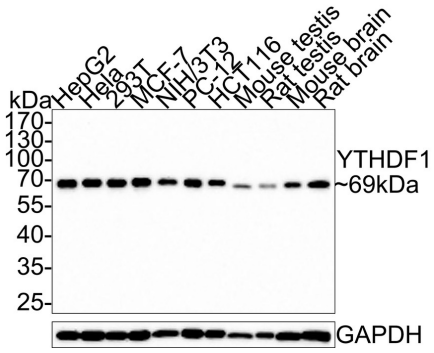

Fig10: Western blot analysis of YTHDF1 on different lysates with Rabbit anti-YTHDF1 antibody (HA721302) at 1/1,000 dilution.

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.

Background References

1. Zhang C, Liu N. N6-methyladenosine (m6A) modification in gynecological malignancies. J Cell Physiol. 2022 Sep;237(9):3465-3479.
2. He L, Li H, Wu A, Peng Y, Shu G, Yin G. Functions of N6-methyladenosine and its role in cancer. Mol Cancer. 2019 Dec 4;18(1):176.