Anti-Lin28A Antibody [A4-A6]

M1301-1

Product Type:	Mouse monoclonal IgG2b, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 23 kDa
Clone number:	A4-A6

Description: LIN28A is conserved, developmentally regulated RNA binding proteins that inhibit the processing and maturation of the let-7 family of miRNAs. LIN28A is localized to the periendoplasmic reticulum (ER) area and inhibits translation of mRNAs that are destined for the ER, reducing the synthesis of transmembrane proteins, ER or Golgi lumen proteins, and secretory proteins. Overexpression of LIN28A, in conjunction with Oct-4, Sox2, and Nanog, can reprogram human fibroblasts to pluripotent, ES-like cells.

Immunogen: Recombinant protein within human Lin28A aa 1-209/209.

Positive control: NCCIT cell lysate, JAR cell lysate, F9 cell lysates, NCCIT, F9, mouse heart tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: Q9H9Z2 Human | Q8K3Y3 Mouse

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:100-1:250
IHC-P	1:200
FC	1:1,000

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

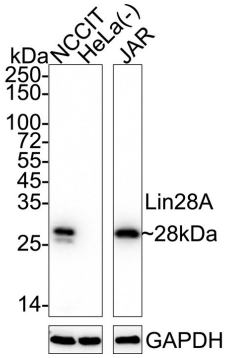

Fig1: Western blot analysis of Lin28A on different lysates with Mouse anti-Lin28A antibody (M1301-1) at 1/1,000 dilution.

Lane 1: NCCIT cell lysate
Lane 2: HeLa cell lysate (negative)
Lane 3: JAR cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 23 kDa
Observed band size: 28 kDa

Exposure time: Lane 1-2: 9 seconds; Lane 3: 3 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 (M1301-1) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

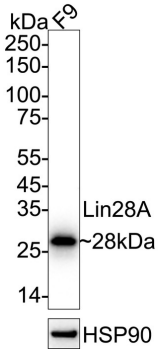

Fig2: Western blot analysis of Lin28A on F9 cell lysates with Mouse anti-Lin28A antibody (M1301-1) at 1/2,000 dilution.

Lysates/proteins at 20 µg/Lane.

Predicted band size: 23 kDa
Observed band size: 28 kDa

Exposure time: 10 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 (M1301-1) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig3: Immunocytochemistry analysis of NCCIT cells labeling Lin28A with Mouse anti-Lin28A antibody (M1301-1) at 1/250 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 Mouse anti-Lin28A antibody (M1301-1) at 1/250 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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 (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

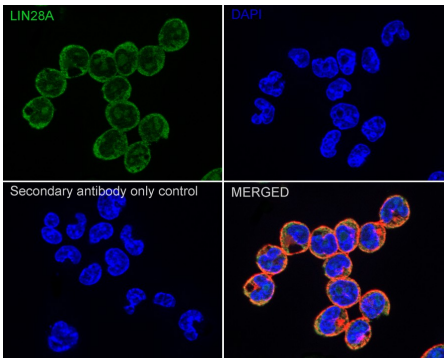

Fig4: Immunocytochemistry analysis of F9 cells labeling Lin28A with Mouse anti-Lin28A antibody (M1301-1) at 1/100 dilution.

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 Mouse anti-Lin28A antibody (M1301-1) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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 (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

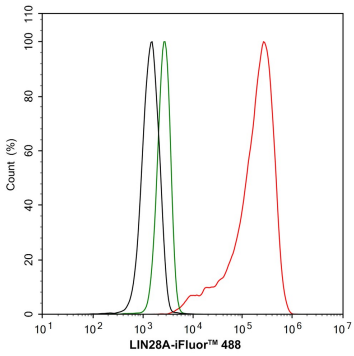

Fig5: Flow cytometric analysis of NCCIT cells labeling Lin28A.

Cells were fixed and permeabilized. Then stained with the primary antibody (M1301-1, 1/1,000) (red) compared with Mouse 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-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

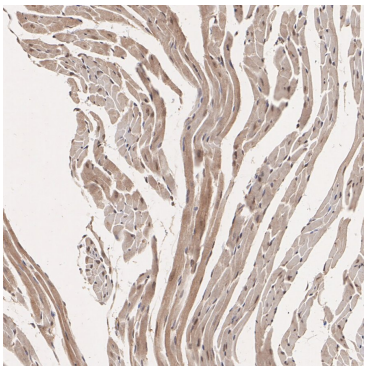

Fig6: Immunohistochemical analysis of paraffin-embedded mouse heart tissue with Mouse anti-Lin28A antibody (M1301-1) at 1/200 dilution.

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 (M1301-1) 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.

Background References

1. "Structural basis of pre-let-7 miRNA recognition by the zinc knuckles of pluripotency factor Lin28."Loughlin F.E., Gebert L.F., Towbin H., Brunschweiger A., Hall J., Allain F.H.Nat. Struct. Mol. Biol. 19:84-89(2012)
2. "LIN28A Is a Suppressor of ER-Associated Translation in Embryonic Stem Cells" Jun Cho, Hyeshik Chang, S. Chul Kwon, Baekgyu Kim, Minju Ha, Yoon Ki Kim, V. Narry Kim. CELL. 151(4)765-777(2012)