Anti-NM23 Antibody [12A1-R]

HA601190

Product Type:	Recombinant Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 17 kDa
Clone number:	12A1-R

Description: Major role in the synthesis of nucleoside triphosphates other than ATP. The ATP gamma phosphate is transferred to the NDP beta phosphate via a ping-pong mechanism, using a phosphorylated active-site intermediate. Possesses nucleoside-diphosphate kinase, serine/threonine-specific protein kinase, geranyl and farnesyl pyrophosphate kinase, histidine protein kinase and 3'-5' exonuclease activities. Involved in cell proliferation, differentiation and development, signal transduction, G protein-coupled receptor endocytosis, and gene expression. Required for neural development including neural patterning and cell fate determination. During GZMA-mediated cell death, works in concert with TREX1. NME1 nicks one strand of DNA and TREX1 removes bases from the free 3' end to enhance DNA damage and prevent DNA end reannealing and rapid repair. Mutations in this gene have been identified in aggressive neuroblastomas. Two transcript variants encoding different isoforms have been found for this gene. Co-transcription of this gene and the neighboring downstream gene (NME2) generates naturally-occurring transcripts (NME1-NME2), which encodes a fusion protein comprised of sequence sharing identity with each individual gene product.

Immunogen:	Synthetic peptide within Human NM23 aa 78-126 / 152.
Positive control:	MCF7 cell lysate, PC-3M cell lysate, HeLa cell lysate, HepG2 cell lysate, A549 cell lysate, NIH/3T3 cell lysate, C6 cell lysate, rat brain tissue lysate, MCF7, human breast carcinoma tissue, human colon tissue, human lymphoma tissue, mouse kidney tissue, rat kidney tissue.
Subcellular location:	Cytoplasm, Nucleus.
Database links:	SwissProt: P15531 Human | P15532 Mouse | Q05982 Rat
Recommended Dilutions:	
WB	1:1,000-1:2,000
IF-Cell	1:100
IHC-P	1:1,000
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

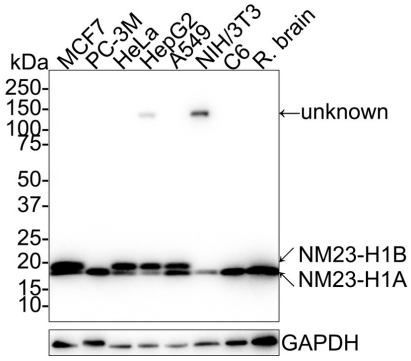

Fig1: Western blot analysis of NM23 on different lysates with Mouse anti-NM23 antibody (HA601190) at 1/1,000 dilution.

Lane 1: MCF7 cell lysate (20 µg/Lane)
Lane 2: PC-3M cell lysate (20 µg/Lane)
Lane 3: HeLa cell lysate (20 µg/Lane)
Lane 4: HepG2 cell lysate (20 µg/Lane)
Lane 5: A549 cell lysate (20 µg/Lane)
Lane 4: NIH/3T3 cell lysate (20 µg/Lane)
Lane 5: C6 cell lysate (20 µg/Lane)
Lane 5: Rat brain tissue lysate (40 µg/Lane)

Predicted band size: 17 kDa
Observed band size: 17/19 kDa

Exposure time: 1 minute;

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 (HA601190) 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.

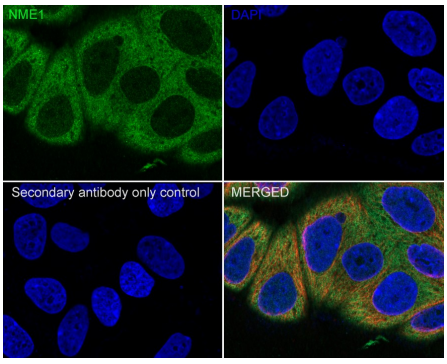

Fig2: Immunocytochemistry analysis of MCF7 cells labeling NM23 with Mouse anti-NM23 antibody (HA601190) at 1/100 dilution.

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 Mouse anti-NM23 antibody (HA601190) 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.

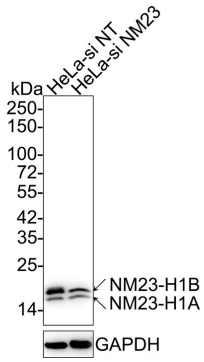

Fig3: Western blot analysis of NM23 on different lysates with Mouse anti-NM23 antibody (HA601190) at 1/2,000 dilution.

Lane 1: HeLa-si NT cell lysate
Lane 2: HeLa-si NM23 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 17 kDa
Observed band size: 17/19 kDa

Exposure time: 46 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 (HA601190) 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.

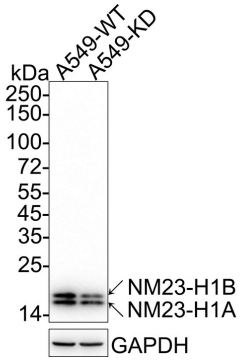

Fig4: Western blot analysis of NM23 on different lysates with Mouse anti-NM23 antibody (HA601190) at 1/2,000 dilution.

Lane 1: A549-si NT cell lysate
Lane 2: A549-si NM23 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 17 kDa
Observed band size: 17/20 kDa

Exposure time: 17 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 (HA601190) at 1/2,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

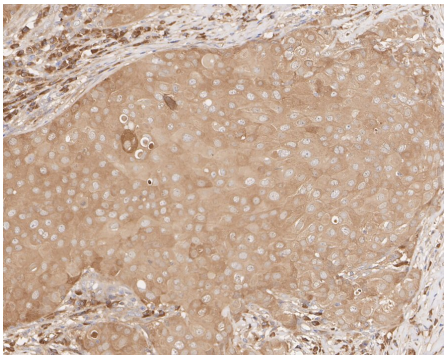

Fig5: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Mouse anti-NM23 antibody (HA601190) at 1/1,000 dilution.

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 (HA601190) 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.

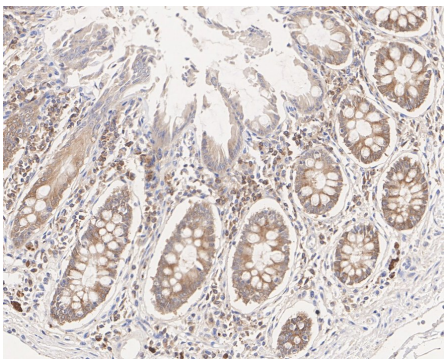

Fig6: Immunohistochemical analysis of paraffin-embedded human colon tissue with Mouse anti-NM23 antibody (HA601190) at 1/1,000 dilution.

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 (HA601190) 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.

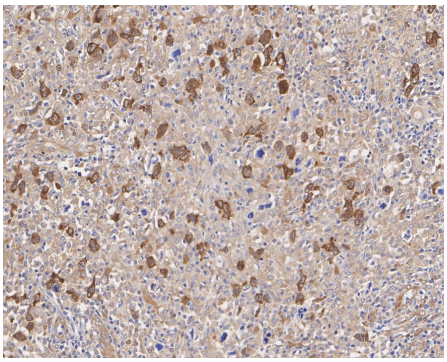

Fig7: Immunohistochemical analysis of paraffin-embedded human lymphoma tissue with Mouse anti-NM23 antibody (HA601190) at 1/1,000 dilution.

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 (HA601190) 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.

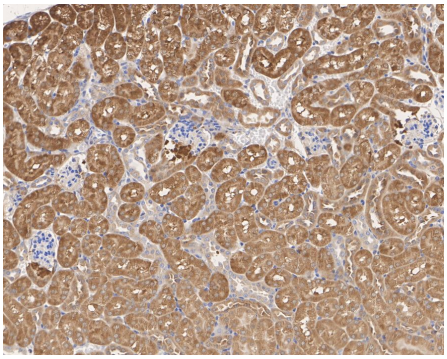

Fig8: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Mouse anti-NM23 antibody (HA601190) at 1/1,000 dilution.

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 (HA601190) 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.

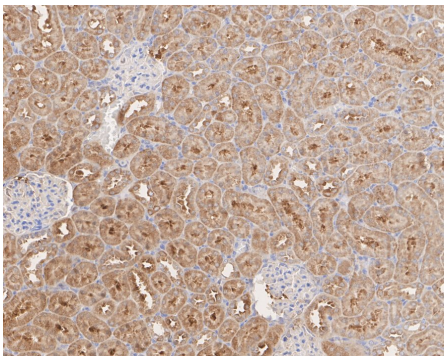

Fig9: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Mouse anti-NM23 antibody (HA601190) at 1/1,000 dilution.

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 (HA601190) 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.

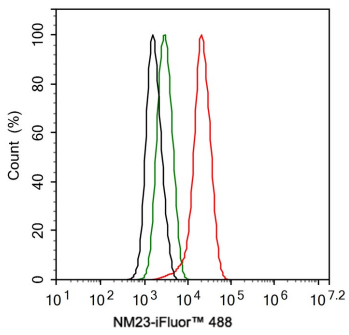

Fig10: Flow cytometric analysis of MCF7 cells labeling NM23.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA601190, 1μg/mL) (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).

Background References

1. Chowdhury D. et al. The exonuclease TREX1 is in the SET complex and acts in concert with NM23-H1 to degrade DNA during granzyme A-mediated cell death. Mol. Cell 23:133-142(2006).
2. Fan Z. et al. Tumor suppressor NM23-H1 is a granzyme A-activated DNase during CTL-mediated apoptosis, and the nucleosome assembly protein SET is its inhibitor. Cell 112:659-672(2003).