Anti-NM23 Antibody [13C1]

EM1901-09



Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, FC, IF-Cell
Molecular Wt:	Predicted band size: 17 kDa
Clone number:	13C1
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.
lmmunogen:	Synthetic peptide within Human NM23 aa 1-50 / 152.
Positive control:	HEK-293 cell lysate, MCF7 cell lysate, Jurkat cell lysate, HeLa cell lysate, HepG2 cell lysate, K-562 cell lysate, A549 cell lysate, Raji cell lysate, NIH/3T3 cell lysate, C6 cell lysate, mouse kidney tissue lysate, mouse liver tissue lysate, mouse brain tissue lysate, rat brain tissue lysate, rat liver tissue lysate, human liver carcinoma tissue, human thyroid tissue, human skin tissue, human breast carcinoma tissue, 293.
Subcellular location:	Nucleus, Cytoplasm.
Database links:	SwissProt: P15531 Human P15532 Mouse Q05982 Rat
Recommended Dilutions: WB IHC-P FC IF-Cell	1:500-1:2,000 1:50-1:100 1:50-1:100 1:100
Storage Buffer:	1*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Store at +4 $^\circ\!\!{\rm C}$ after thawing. Aliquot store at -20 $^\circ\!\!{\rm C}$. Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

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Images



kDa<u>WC</u> kDa<u>WC</u> 250-150-100-55-42-35-25-14-NM23 **Fig1:** Western blot analysis of NM23 on different lysates with Mouse anti-NM23 antibody (EM1901-09) at 1/2,000 dilution.

Lane 1: HEK-293 cell lysate (20 µg/Lane) Lane 2: MCF7 cell lysate (20 µg/Lane) Lane 3: Jurkat cell lysate (20 µg/Lane) Lane 4: HeLa cell lysate (20 µg/Lane) Lane 5: HepG2 cell lysate (20 µg/Lane) Lane 6: K-562 cell lysate (20 µg/Lane) Lane 7: A549 cell lysate (20 µg/Lane) Lane 8: Raji cell lysate (20 µg/Lane)

Predicted band size: 17 kDa Observed band size: 17/20 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 (EM1901-09) at 1/2,000 dilution was used in 5% NFDM/TBST at 4° C 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 NM23 on different lysates with Mouse anti-NM23 antibody (EM1901-09) at 1/2,000 dilution.

- Lane 1: NIH/3T3 cell lysate (20 µg/Lane)
- Lane 2: C6 cell lysate (20 µg/Lane)
- Lane 3: Mouse kidney tissue lysate (40 µg/Lane)
- Lane 4: Mouse liver tissue lysate (40 µg/Lane)
- Lane 5: Mouse brain tissue lysate (40 µg/Lane)
- Lane 6: Rat brain tissue lysate (40 µg/Lane)
- Lane 7: Rat liver tissue lysate (40 µg/Lane)

Predicted band size: 17 kDa Observed band size: 17 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 (EM1901-09) at 1/2,000 dilution was used in 5% NFDM/TBST at 4° C overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

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Fig3: Immunohistochemical analysis of paraffin-embedded human liver cancer tissue with Mouse anti-NM23 antibody (EM1901-09) 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 ddH₂O and PBS, and then probed with the primary antibody (EM1901-09) 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.



Fig4: Immunohistochemical analysis of paraffin-embedded human thyroid tissue using anti-NM23 antibody. The section was pretreated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (EM1901-09, 1/50) for 30 minutes 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.

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Fig5: Immunohistochemical analysis of paraffin-embedded human skin tissue with Mouse anti-NM23 antibody (EM1901-09) 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 ddH₂O and PBS, and then probed with the primary antibody (EM1901-09) 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.



Fig6: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-NM23 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (EM1901-09, 1/50) for 30 minutes 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.

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Fig7: Flow cytometric analysis of NM23 was done on 293 cells. The cells were fixed, permeabilized and stained with the primary antibody (EM1901-09, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Mouse IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Fig8: Immunocytochemistry analysis of MCF7 cells labeling NM23 with Mouse anti-NM23 antibody (EM1901-09) 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 (EM1901-09) at 1/100 dilution in 1% BSA in PBST overnight at 4 $^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluorTM 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 $+4^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor 1594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

Fig9: Western blot analysis of NM23 on different lysates with Mouse anti-NM23 antibody (EM1901-09) at 1/2,000 dilution.

Lane 1: A549-si NT cell lysate (10 µg/Lane) Lane 2: A549-si NM23 cell lysate (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 (EM1901-09) 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.

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

Background References

- 1. 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).
- 2. 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).

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

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