Human nm23-H1 Rabbit Polyclonal Antibody

Cat# CY-P1032

50 µg (0.5 mg/mL x 100 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
	WB	H	29 kDa	Rabbit IgG

Background

The nm23 family of genes is characterized by their reduced expression in certain highly metastatic cell lines and tumors (1). In humans, the eight nm23 genes that have been identified to date, nm23-H1, nm23-H2, nm23-H3, nm23-H4, nm23-H5, nm23-H6, nm23-H7, and nm23-H8, encode nucleoside diphosphate kinases (NDP kinases), or homologous isoforms (2). However, while nm23-H1 was initially identified as a putative metastasis suppressor, its enzymatic activity does not appear to be responsible for its function as a metastasis suppressor during tumor progression (3). Studies of nm23 family proteins in other species have provided evidence for their role in proliferation, differentiation, apoptosis, development, and endocytosis (4). The ability of nm23 family proteins to regulate a diverse set of cellular processes has recently been linked to their ability to modulate signal transduction by a diverse set of growth factors, such as transforming growth factor-β1 (TGF-β1), nerve growth factor (NGF), platelet-derived growth factor (PDGF), and insulin-like growth factor-1 (5).

The most widely expressed isoforms, nm23-H1 and -H2 also regulate a diverse array of cellular events including growth and development, tumour metastasis and transcriptional regulation (6,7). Despite sharing 88% amino acid sequence identity, these two isoforms are reported to have distinct cellular functions (8). Accumulating evidence indicates that protein–protein interactions modulate the specific molecular actions of NDP kinase, with new binding partners being identified at an increasing rate (7).

Whereas reduced expression of nm23 is associated with a high potential for metastasis in some tumor types, its expression is increased in aggressive neuroblastoma (9). In addition, immunohistochemical studies have reported that patients with high-grade non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma exhibited significantly higher levels of nm23-H1 expression than did those with low-grade NHL (10). The serum nm23-H1 level is significantly higher in patients with indolent and aggressive non-Hodgkin lymphoma, and acute myelogenous leukemia (AML) than in normal controls (11, 12).

Specificity/Sensitivity: Human nm23-H1 Antibody detects endogenous levels of nm23-H1 protein.

Source/Purification: Polyclonal antibody is produced by immunizing rabbit with a full length recombinant human nm23-H1. IgG is purified by immunoaffinity chromatography.

Recommended Antibody Dilutions: Western blotting: 0.5-1 µg/mL.

Storage: Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at -20°C.

Applications Key: WB: Western **IP:** Immunoprecipitation **IHC:** Immunohistochemistry **IC:** Immunocytochemistry **F:** Flow cytometry **E:** ELISA **FP:** Fluorescence Polarization assay
Species Cross-Reactivity Key: H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Mi:** mink **C:** chicken **X:** *Xenopus* **Z:** zebra fish **All:** all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

General References:

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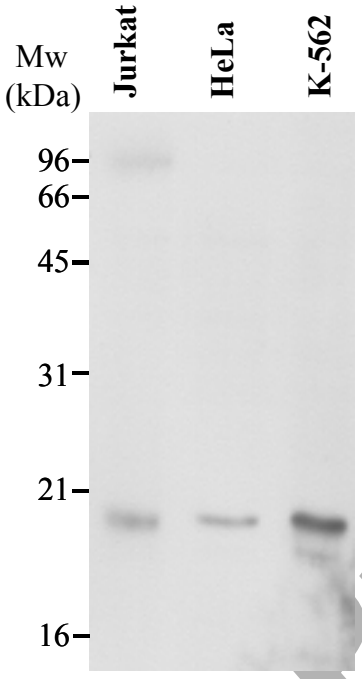
Fig.1 Western blot analysis of Human nm23-H1



Western Immunoblotting Protocol

Solutions and Reagents
Note: Prepare solutions with Milli-Q or equivalently purified water.

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
SDS Sample Buffer (1X): 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 mL, add 15 mL 10X TBS to 135 mL water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 mL Tween-20 (100%).
10X TBS (Tris-buffered saline): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20 with 5% blocking agent for 20 mL, add 2 mL 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µL Tween-20 (100%).
Chemiluminescent HRP Detection: secondary anti-rabbit IgG antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)
Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20
Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Protein Blotting
A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.

2. Aspirate media from cultures; wash cells with 1X PBS, aspirate.
3. Lyse cells by adding 1X SDS Sample Buffer (100 µL per well of 6-well plate or 500 µL per plate of 10 cm2 plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µL sample to 95–100°C for 5 minutes, cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µL onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose membrane.

Membrane Blocking and Antibody Incubations

Note: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 mL TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 mL of Blocking Buffer for 1 hour at room temperature.
3. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 mL Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
5. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3000 in 10 mL of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 mL of TBS/T.

Detection of Proteins

1. Incubate membrane with 4 mL ECL™ with gentle agitation for 1 minute at room temperature.
2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial ten-second exposure should indicate the proper exposure time.

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