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NPM1 Polyclonal Antibody

Catalog Number **PA1-029**

Product data sheet

Details	
Size	100 µg
Host/Isotope	Rabbit / IgG
Class	Polyclonal
Type	Antibody
Immunogen	Synthetic peptide corresponding to residues 23-38 and 226-240 of human NPM.
Conjugate	Unconjugated
Form	Liquid
Concentration	1 mg/mL
Purification	Antigen affinity chromatography
Storage buffer	PBS with 1mg/mL BSA
Contains	0.05% sodium azide
Storage Conditions	-20° C, Avoid Freeze/Thaw Cycles

Species Reactivity	
Species reactivity	Human, Mouse, Non-human primate, Rat

Tested Applications	Dilution *
Immunohistochemistry (Paraffin) (IHC (P))	1:200
Western Blot (WB)	1:500-1:1,000
Immunocytochemistry (ICC/IF)	1:100

* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Product specific information

PA1-029 detects nucleophosmin (NPM) from human, rat, and mouse samples. PA1-029 has been successfully used in Western blot and immunofluorescence procedures. By Western blot, this antibody detects an ~ 38 kDa protein corresponding to human, mouse, and rat nucleophosmin in various cell lysates. The PA1-029 immunogen is a synthetic peptide corresponding to amino acid residues 23-38 and 226-240 from human NPM.

Background/Target Information

NPM1 (Nucleophosmin 1, B23, nutramin, NO38) is a ubiquitously expressed phosphoprotein involved in ribosome assembly/transport, cytoplasmic /nuclear trafficking, regulation of DNA polymerase alpha activity, centrosome duplication, and regulation of p53. NPM1 continuously shuttles between the nucleus, cytoplasm, nucleolus and chaperoning core histones from the nucleus to the cytoplasm. NPM1 has been shown to bind nucleic acid, prevent protein aggregation via its chaperon activities, protect enzymes during thermal denaturation, and facilitate renaturation of chemically denatured proteins. In its cellular structure role, there is evidence suggesting NPM1 is associated with the centrosome, and is the substrate of CDK2/cyclin E during duplication of centrosomes (cellular division). Due to the NPM1 gene interaction with several tumor-associated chromosome translocations, NPM1 is thought to be a portion of several fusion proteins: NPM-ALK, NPM-RAR, and NPM-MLF1. While it is not thought to be part of the transforming potential of these fusion proteins, NPM1 is believed to act as the interface for oligomerization and oncogenic conversion of these tumor promoting fusion proteins. Further, NPM1 is also known to sequester the tumor suppressor RF in the nucleolus, protecting it from degradation until it is necessary. Dysfunction of the NPM1 protein is associated with diseases such as acute myeloid leukemia and lymphomatoid papulosis.

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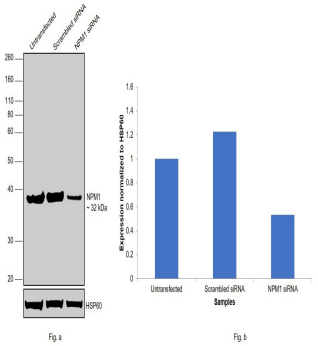
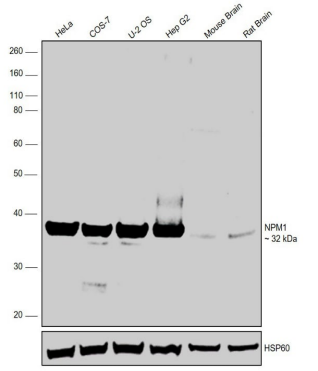
Product Images For NPM1 Polyclonal Antibody



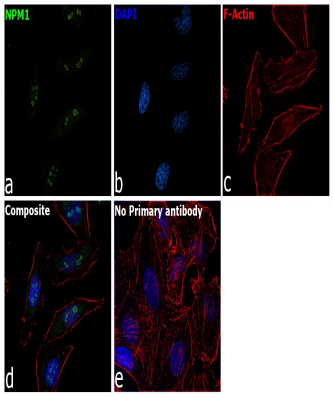
NPM1 Antibody (PA1-029)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. HeLa cells were transfected with NPM1 siRNA and decrease in signal intensity was observed in western blot application using Anti-NPM1 Polyclonal Antibody (Product # PA1-029). {KD}



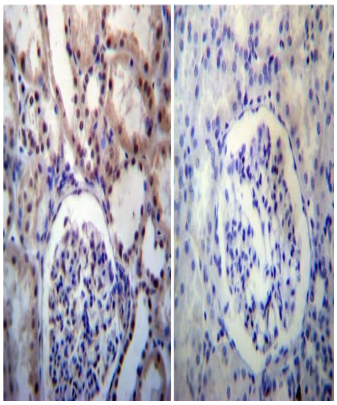
NPM1 Antibody (PA1-029) in WB

Western blot was performed using Anti-NPM1 Polyclonal Antibody (Product # PA1-029) and a 32 kDa band corresponding to NPM1 was observed across all the cell lines and tissues tested. Modified whole cell extracts (1% SDS) (30 µg lysate) of HeLa (Lane 1), COS-7 (Lane 2), U-2OS (Lane 3), Hep G2 (Lane 4), tissue extracts (30 µg lysate) of Mouse Brain (Lane 5) and Rat Brain (Lane 6) were electrophoresed using NuPAGE™ 10% Bis-Tris Protein Gel (Product # NP0302BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (1:750 dilution) and detected by chemiluminescence with Goat anti-Rabbit IgG (Heavy Chain), Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:4,000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005).



NPM1 Antibody (PA1-029) in ICC/IF

Immunofluorescence analysis of NPM1 was performed using 70% confluent log phase HeLa cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 2% BSA for 1 hour at room temperature. The cells were labeled with NPM1 Polyclonal Antibody (Product # PA1-029) at 1:100 dilution in 0.1% BSA, incubated at 4 degree Celsius overnight and then with Goat anti-Rabbit IgG (Heavy Chain), Superclonal™ Recombinant Secondary Antibody, Alexa Fluor 488 conjugate (Product # A27034) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with ProLong™ Diamond Antifade Mountant with DAPI (Product # P36962). F-actin (Panel c: red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing staining in the nucleolus. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



NPM1 Antibody (PA1-029) in IHC (P)

Immunohistochemistry was performed on normal deparaffinized human Kidney tissue. To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:500 with a rabbit polyclonal antibody recognizing Nucleophosmin (Product # PA1-029) or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP, followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.

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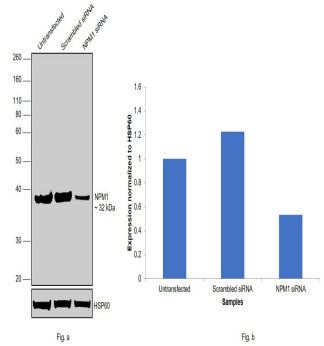
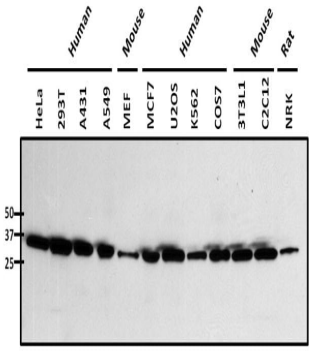


NPM1 Antibody (PA1-029) in WB

Knockdown of NPM1 was achieved by transfecting HeLa with NPM1 specific siRNAs (Silencer® select Product # s9677, s9676). Western blot analysis (Fig. a) was performed using modified whole cell extracts (1% SDS) from NPM1 knockdown cells (Lane 3), non-specific scrambled siRNA transfected cells (Lane 2) and untransfected cells (Lane 1). The blot was probed with NPM1 Polyclonal Antibody (Product # PA1-029, 1:750 dilution) and Goat anti-Rabbit IgG (Heavy Chain), Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 0.25 µg/mL, 1:4,000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig. b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to NPM1.



NPM1 Antibody (PA1-029) in WB

Western blot analysis of Nucleophosmin (Product # PA1-029) was performed by loading 25 µg of various whole cell lysates onto a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked with 5% Milk/TBST for at least 1 hour. Membranes were probed with a rabbit polyclonal antibody recognizing Nucleophosmin at a dilution of 1:500 overnight at 4°C on a rocking platform. Membranes were washed in TBS-0.1% Tween 20 and probed with a goat anti-rabbit-HRP secondary antibody (Product # 31460) at a dilution of 1:20,000 for at least one hour. Membranes were washed and chemiluminescent detection performed using Pierce Super Signal West Pico (Product # 34077).

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