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NPM1 Polyclonal Antibody

Catalog Number PA5-17742

Product data sheet

Details		Species Reactivity	
Size	100 µL	Species reactivity	Human, Mouse, Non-human primate, Rat
Host/Isotope	Rabbit / IgG		primate, ivat
Class	Polyclonal	Tested Applications	Dilution *
Туре	Antibody	ChIP assay (ChIP)	2.5 µg/10^6 cells
Immunogen	Synthetic peptide corresponding to residues surrounding the amino-terminus of human NPM	Western Blot (WB)	1:1,000-1:1,200
		Immunocytochemistry (ICC/IF)	1:100
Conjugate	Unconjugated	* 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.	
Form	Liquid		
Concentration	21 µg/mL		
Purification	Antigen affinity chromatography		
Storage buffer	0.01M HEPES, pH 7.5, with 0.15M NaCl, 100μg/mL BSA, 50% glycerol		
Contains	no preservative		
Storage Conditions	-20°C		

Product specific information

It is not recommended to aliquot this antibody.

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.

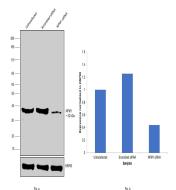
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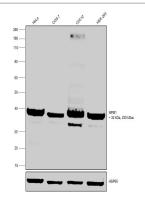
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Product Images For NPM1 Polyclonal Antibody



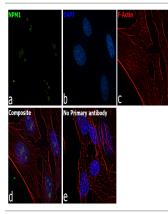
NPM1 Antibody (PA5-17742)

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 # PA5-17742). {KD}



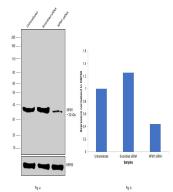
NPM1 Antibody (PA5-17742) in WB

Western blot was performed using Anti-NPM1 Polyclonal Antibody (Product # PA5-17742) and 32 kDa and 230 kDa bands corresponding to NPM1 was observed across all the cell lines tested. Modified whole cell extracts (1% SDS) (30 µg lysate) of HeLa (Lane 1), COS-7 (Lane 2), C2C12 (Lane 3) and HEK-293 (Lane 4) 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 (1:1200 dilution) and detected by chemiluminescence with Goat anti-Rabbit IgG (Heavy Chain), Superclonal[™] Recombinant Secondary Antibody, HRP (Product # A27036, 1:4000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005).



NPM1 Antibody (PA5-17742) 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 10 minutes, and blocked with 2% BSA for 10 minutes at room temperature. The cells were labeled with NPM1 Polyclonal Antibody (Product # PA5-17742) at 1:100 dilution in 0.1% BSA, incubated at 4 degree Celsius overnight and then labeled with Goat anti-Rabbit IgG (Heavy Chain) Superclonal[™] Recombinant Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A27034, 1:2000 dilution) 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 nucleolar localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



NPM1 Antibody (PA5-17742) 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 # PA5-17742, 1:1200 dilution) and Goat anti-Rabbit IgG (Heavy Chain), Superclonal[™] Recombinant Secondary Antibody, HRP (Product # A27036, 1:4000 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.

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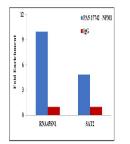
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NPM1 Antibody (PA5-17742) in ChIP



Chromatin Immunoprecipitation (ChIP) assay of endogenous NPM1 protein using Anti-NPM1 Antibody: ChIP was performed using Anti-NPM1 Rabbit polyclonal Antibody (Product # PA5-17742, 5 µg) on sheared chromatin from HeLa cells using the MAGnify ChIP System kit (Product # 49-2024). Normal Rabbit IgG was used as a negative IP control. The purified DNA was analyzed by qPCR using primers binding to 45s rDNA gene body (+13kb) and SAT2 satellite repeats. Data is presented as fold enrichment of the antibody signal versus the negative control IgG using the comparative CT method.

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