

NPM1 Monoclonal Antibody (FC-61991)

Catalog Number32-5200

Product data sheet

Details		Species Reactivity	
Size	100 µg	Species reactivity	Human, Mouse, Rat
Host/Isotope	Mouse / IgG1	Published species	Virus, Non-human primate, Human, Mouse, Not Applicable
Class	Monoclonal		
Type	Antibody	Tested Applications	
Clone	FC-61991	ELISA (ELISA)	1-2 µg/mL
Immunogen	NPM/B23 purified from rat hepatoma.	Immunohistochemistry (IHC)	Assay-dependent
Conjugate	Unconjugated	Immunoprecipitation (IP)	10 µg
Form	Liquid	Western Blot (WB)	1-3 µg/mL
Concentration	0.5 mg/mL	RNA Immunoprecipitation (RIP)	Assay-dependent
Purification	Protein A	Immunocytochemistry (ICC/IF)	2-10 µg/mL
Storage buffer	PBS, pH 7.4	Published Applications	
Contains	0.1% sodium azide	Immunohistochemistry (IHC)	See 6 publications below
Storage Conditions	-20°C	Immunocytochemistry (ICC/IF)	See 24 publications below
		Western Blot (WB)	See 22 publications below
		Miscellaneous PubMed (Misc)	See 19 publications below
		Immunoprecipitation (IP)	See 3 publications below

* 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

This antibody reacts with the C-terminus of Nucleophosmin (B23). Positive controls used: HeLa, K562, Jurkat, MCF-7, and HL60 cell lysates). In western blots the antibody recognizes a band at ~37 kDa.

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.

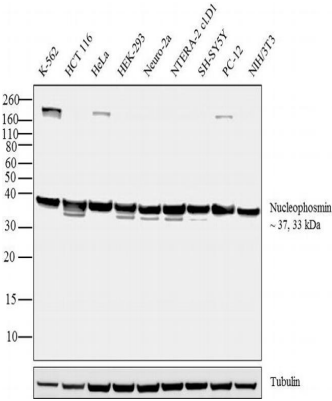
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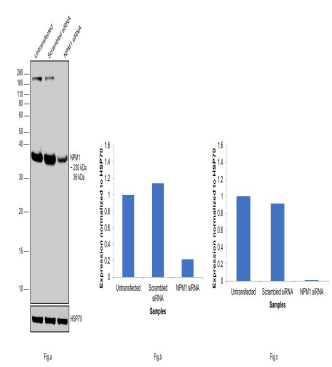
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Product Images For NPM1 Monoclonal Antibody (FC-61991)



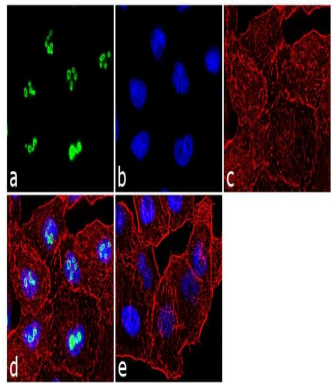
NPM1 Antibody (32-5200) in WB

Western blot analysis was performed on nuclear enriched cell extracts of K-562 (Lane 1), HCT 116 (Lane 2), HeLa (Lane 3), HEK-293 (Lane 4), Neuro-2a (Lane 5), NTERA-2 cl.D1 (Lane 6), SH-SY5Y (lane 7), PC-12 (lane 8) and NIH/3T3 (Lane 9). Blots were probed with Anti- Nucleophosmin Mouse Monoclonal Antibody (Product # 32-5200, 2 µg/mL) and detected by chemiluminescence using Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conj µgate (Product # A28177, 0.4 µg/mL, 1:2500 dilution). A ~ 37 kDa band corresponding to Nucleophosmin was observed across all cell lines. An additional ~ 33 kDa band corresponding to Nucleophosmin isoform was observed in HCT 116 (Lane 2), HEK-293 (Lane 4), Neuro-2a (Lane 5), NTERA-2 cl.D1 (Lane 6) and SH-SY5Y (lane 7). Bands of ~210 kDa observed in K-562 (Lane 1), HeLa (Lane 3) and PC-12 (lane 8) could be due to oligomerisation of Nucleophosmin. Protein samples were electrophoresed using Novex® NuPAGE® 4-12 % Bis-Tris gel (Product # NP0321BOX), XCell SureLock™ Electrophoresis System (Product # EI0002) and Novex® Sharp Pre-Stained Protein Standard (Product # LC5800). Resolved proteins were transferred onto a nitrocellulose membrane and probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).



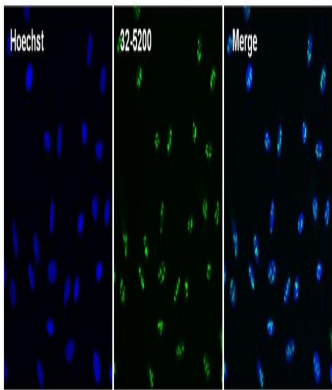
NPM1 Antibody (32-5200)

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 Monoclonal Antibody (FC-61991) (Product # 32-5200). {KD}



NPM1 Antibody (32-5200) in ICC/IF

Immunofluorescence analysis of Nucleophosmin was performed using 70% confluent log phase A549 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with Nucleophosmin (FC-61991) Mouse Monoclonal Antibody (Product # 32-5200) at 2 µg/mL in 0.1% BSA and incubated for 3 hours at room temperature and then labeled with Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conj µgate (Product # A28175) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Alexa Fluor® 555 Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing nucleolar localization. Panel e shows the no primary antibody control. The images were captured at 60X magnification.



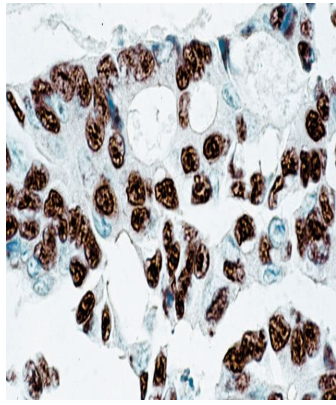
NPM1 Antibody (32-5200) in ICC/IF

Immunofluorescent analysis of Nucleophosmin (green) in HeLa cells. The cells were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes, and blocked with 3% Blocker BSA (Product # 37525) in PBS for 30 minutes at room temperature. Cells were stained with a Nucleophosmin monoclonal antibody (Product # 32-5200) at a concentration of 10 µg/mL for 1 hour at room temperature, and then incubated with a Goat anti-Mouse IgG (H+L) Superclonal Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A28175) at a dilution of 1:1000 for 1 hour at room temperature (green). Nuclei (blue) were stained with Hoechst 33342 dye (Product # 62249). Images were taken on a Thermo Scientific ToxInsight at 20X magnification.

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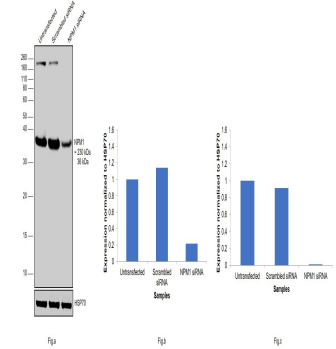
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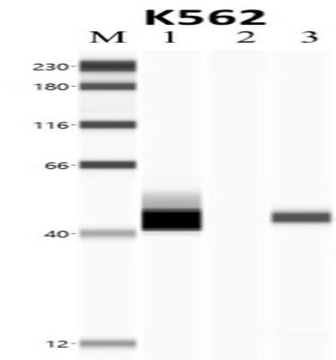
NPM1 Antibody (32-5200) in IHC

Immunohistochemical staining of human colon cancer tissue using Mouse anti-Nucleophosmin monoclonal antibody (clone FC-61991) (Product # 32-5200).



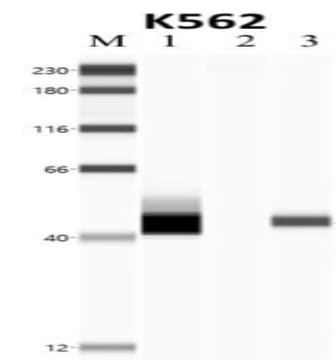
NPM1 Antibody (32-5200) in WB

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 the NPM1 knockdown cells (Lane 3), non-specific scrambled siRNA transfected cells (Lane 2) and untransfected cells (Lane 1). The blot was probed with NPM1 Monoclonal Antibody (FC-61991) (Product # 32-5200, 0.5 µg/mL) and Goat anti-Rabbit IgG (Heavy Chain), Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:4,000 dilution). Densitometric analysis of this western blot for the 36 kDa band is shown in Fig. b and for the 230 kDa band in Fig. c. Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to NPM1.



NPM1 Antibody (32-5200) in IP

Immunoprecipitation of NPM1 was performed on K562 cells. Antigen-antibody complexes were formed by incubating approximately 500 µg whole cell lysate with 5 µg of NPM1 monoclonal antibody (Product # 325200) rotating 60 min at RT. The immune complexes were captured on 625 µg of anti-mouse coated Dynabeads (Product # 11202D) and washed extensively. They were then eluted and analyzed using the Jess Simple Western system. Lane 1 is input, lane 2 IP without antibody and lane 3 IP with antibody. Target was detected a NPM1 monoclonal antibody (Product # 325200) at a dilution of 1:25, followed by a 1:100 dilution of secondary antibody. Data courtesy of the Yeo lab as part of the ENCODE project (www.encodeproject.org).



NPM1 Antibody (32-5200) in RIP

RNA immunoprecipitation (RIP) western of NPM1 was performed on K562 cells. Antigen-antibody complexes were formed by incubating approximately 500 µg whole cell lysate with 5 µg of NPM1 monoclonal antibody (Product # 325200) rotating 60 min at RT. The immune complexes were captured on 625 µg of anti-mouse coated Dynabeads (Product # 11202D) and washed extensively. They were then eluted and analyzed using the Jess Simple Western system. Lane 1 is input, lane 2 IP without antibody and lane 3 IP with antibody. Target was detected a NPM1 monoclonal antibody (Product # 325200) at a dilution of 1:25, followed by a 1:100 dilution of secondary antibody. Data courtesy of the Yeo lab as part of the ENCODE project (www.encodeproject.org).

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PubMed References For NPM1 Monoclonal Antibody (FC-61991)

6 Immunohistochemistry References

Species / Dilution	Summary
Human / Not Cited	32-5200 was used in Immunohistochemistry-immunofluorescence to demonstrate that cancer cells respond to cisplatin treatment with the nucleolar accumulation of inorganic polyphosphate (polyP), a universally conserved high-energy compound. Frontiers in oncology (2021; 9:) "Accumulation of Nucleolar Inorganic Polyphosphate Is a Cellular Response to Cisplatin-Induced Apoptosis." Author(s):Xie L,Rajpurkar A,Quarles E,Taube N,Rai AS,Erba J,Sliwinski B,Markowitz M,Jakob U,Knoefler D PubMed Article URL: http://dx.doi.org/10.3389/fonc.2019.01410
Not Applicable / Not Cited	32-5200 was used in immunohistochemistry to review the transcription factors that the re-replication inhibitor Geminin binds BMC biochemistry (2009; 10:) "TIPT2 and geminin interact with basal transcription factors to synergize in transcriptional regulation." Author(s):Pitulescu ME,Teichmann M,Luo L,Kessel M PubMed Article URL: http://dx.doi.org/10.1186/1471-2091-10-16
Human / 1:500	32-5200 was used in Immunohistochemistry-immunofluorescence to show that expansion microscopy enables superresolved imaging of the highly dynamic structure of nuclei in immune cells. Biophysical reports (2023; 3:) "Expansion microscopy of neutrophil nuclear structure and extracellular traps." Author(s):Holsapple JS,Schnitzler L,Rusch L,Baldeweg TH,Neubert E,Kruss S,Erpenbeck L PubMed Article URL: http://dx.doi.org/10.1016/j.bpr.2022.100091
Not Applicable / Not Cited	32-5200 was used in immunohistochemistry to assess the role of nucleophosmin exon-12 mutations in adult acute myeloid leukemia Blood (2006; 108: 1999) "Immunohistochemistry predicts nucleophosmin (NPM) mutations in acute myeloid leukemia." Author(s):Falini B,Martelli MP,Bolli N,Bonasso R,Ghia E,Pallotta MT,Diverio D,Nicoletti I,Pacini R,Tabarrini A,Galletti BV, Mannucci R,Roti G,Rosati R,Specchia G,Liso A,Tiacci E,Alcalay M,Luzi L,Volorio S,Bernard L,Guarini A,Amadori S, Mandelli F,Pane F,Lo-Coco F,Saglio G,Pellicci PG,Martelli MF,Mecucci C PubMed Article URL: http://dx.doi.org/10.1182/blood-2006-03-007013
Mouse / 1:200	32-5200 was used in Immunohistochemistry to describe the generation of an inducible Sod1 knockout in KRAS-driven NSCLC mouse model. Nature communications (2021; 12:) "SOD1 regulates ribosome biogenesis in KRAS mutant non-small cell lung cancer." Author(s):Wang X,Zhang H,Sapio R,Yang J,Wong J,Zhang X,Guo JY,Pine S,Van Remmen H,Li H,White E,Liu C,Kiledjian M,Pestov DG,Steven Zheng XF PubMed Article URL: http://dx.doi.org/10.1038/s41467-021-22480-x
Human / Not Cited	325200 was used in immunohistochemistry to investigate the roles of PARP2 and PARP3 during DNA break repair Nucleic acids research (2017; 45: 2546) "Overlapping roles for PARP1 and PARP2 in the recruitment of endogenous XRCC1 and PNKP into oxidized chromatin." Author(s):Hanzlikova H,Gittens W,Krejciakova K,Zeng Z,Caldecott KW PubMed Article URL: http://dx.doi.org/10.1093/nar/gkw1246

24 Immunocytochemistry References

Species / Dilution	Summary
Human / Not Cited	32-5200 was used in immunocytochemistry to investigate the effect of modulating the rRNA transcription rate on p53 induction in mammalian cells. Oncogene (2011; 30: 3274) "The balance between rRNA and ribosomal protein synthesis up- and downregulates the tumour suppressor p53 in mammalian cells." Author(s):Donati G,Bertoni S,Brighenti E,Vici M,Treré D,Volarevic S,Montanaro L,Derenzini M PubMed Article URL: http://dx.doi.org/10.1038/onc.2011.48
Human / Not Cited Virus / Not Cited	Oncogene (2006; 25: 448) "Hepatitis C virus core protein recruits nucleolar phosphoprotein B23 and coactivator p300 to relieve the repression effect of transcriptional factor YY1 on B23 gene expression." Author(s):Mai RT,Yeh TS,Kao CF,Sun SK,Huang HH,Wu Lee YH PubMed Article URL: http://dx.doi.org/10.1038/sj.onc.1209052

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	32-5200 was used in immunocytochemistry to assess induced Bax-regulated nuclear rupture and bubble budding followed by release of nuclear proteins due to cellular stress
Not Applicable / Not Cited	Nucleus (Austin, Tex.) (2015; 5: 527) "Cellular stress induces Bax-regulated nuclear bubble budding and rupture followed by nuclear protein release." Author(s):Lindenboim L,Sasson T,Worman HJ,Borner C,Stein R PubMed Article URL: http://dx.doi.org/10.4161/19491034.2014.970105
Human / 1:500	32-5200 was used in Immunocytochemistry-immunofluorescence to study the effects of heatshock on epigenetic regulators, revealing that the nucleolus acts as a quality control centre and enables recovery of the epigenome after heatshock. eLife (2019; 8:) "Protein quality control in the nucleolus safeguards recovery of epigenetic regulators after heat shock." Author(s):Azkanaz M,Rodríguez López A,de Boer B,Huiting W,Angrand PO,Vellenga E,Kampinga HH,Bergink S,Martens JH,Schuringa JJ,van den Boom V PubMed Article URL: http://dx.doi.org/10.7554/eLife.45205
Not Applicable / 1:100	32-5200 was used in immunocytochemistry to utilize human oocytes, models of in vitro implantation, and embryos to study expression of placenta-specific 8 Fertility and sterility (2016; 106: 781) "Expression of placenta-specific 8 in human oocytes, embryos, and models of in vitro implantation." Author(s):Li M,Liu D,Wang L,Wang W,Wang A,Yao Y PubMed Article URL: http://dx.doi.org/10.1016/j.fertnstert.2016.05.018
Mouse / 1:2000	32-5200 was used in Immunocytochemistry to introduce new tools for studies of the redox biology of the mammalian nucleolus and identifies pre-rRNA maturation steps sensitive to H ₂ O ₂ stress. Frontiers in molecular biosciences (2021; 8:) "Effects of Hydrogen Peroxide Stress on the Nucleolar Redox Environment and Pre-rRNA Maturation." Author(s):Sapio RT,Burns CJ,Pestov DG PubMed Article URL: http://dx.doi.org/10.3389/fmolb.2021.678488
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Not Applicable / Not Cited	32-5200 was used in immunocytochemistry, immunoprecipitation, and western blot to study translocation of p14(ARF)/p19 (ARF) and B23 into the nucleus Cancer research (2008; 68: 1398) "DNA damage-dependent translocation of B23 and p19 ARF is regulated by the Jun N-terminal kinase pathway." Author(s):Yogev O,Saadon K,Anzi S,Inoue K,Shaulian E PubMed Article URL: http://dx.doi.org/10.1158/0008-5472.CAN-07-2865
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Human / Not Cited	BMC cancer (2010; 10:) "Glycogen Synthase Kinase-3 regulates multiple myeloma cell growth and bortezomib-induced cell death." Author(s):Piazza F,Manni S,Tubi LQ,Montini B,Pavan L,Colpo A,Gnoato M,Cabrelle A,Adami F,Zambello R,Trentin L, Gurrieri C,Semenzato G PubMed Article URL: http://dx.doi.org/10.1186/1471-2407-10-526
Human / 1:1000	32-5200 was used in Immunocytochemistry to analyze in a cellular model the relative toxicity of DPRs and RNA. eLife (2021; 10:) "Multiple pathways of toxicity induced by C9orf72 dipeptide repeat aggregates and G⁴C² RNA in a cellular model." Author(s):Frottin F,Pérez-Berlanga M,Hartl FU,Hipp MS PubMed Article URL: http://dx.doi.org/10.7554/eLife.62718

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	32-5200 was used in immunocytochemistry to study why HSC function declines with age
Mouse / Not Cited	<p>Nature (2014; 512: 198)</p> <p>"Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells."</p> <p>Author(s):Flach J,Bakker ST,Mohrin M,Conroy PC,Pietras EM,Reynaud D,Alvarez S,Diolaiti ME,Ugarte F,Forsberg EC,Le Beau MM,Stohr BA,Méndez J,Morrison CG,Passegué E</p> <p>PubMed Article URL:http://dx.doi.org/10.1038/nature13619</p>
Mouse / Not Cited	<p>32-5200 was used in Immunocytochemistry to demonstrate that MYC is coordinately regulated by cell-autonomous and microenvironmental signals, and establish CAF-derived FGF1 as a novel paracrine regulator of oncogenic transcription.</p>
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Human / Not Cited

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"Involvement of Nucleophosmin (NPM1/B23) in Assembly of Infectious HPV16 Capsids."
Author(s):Day PM,Thompson CD,Pang YY,Lowy DR,Schiller JT
PubMed Article URL:<http://dx.doi.org/10.1016/j.pvr.2015.06.005>

Non-human primate / 1.5 µg /ml

Molecular and cellular biology (2005; 25: 1258)
"Nucleophosmin (B23) targets ARF to nucleoli and inhibits its function."

Human / 1.5 µg/ml

Author(s):Korgaonkar C,Hagen J,Tompkins V,Frazier AA,Allamargot C,Quelle FW,Quelle DE

Mouse / 1.5 µg/ml

PubMed Article URL:<http://dx.doi.org/10.1128/MCB.25.4.1258-1271.2005>

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