abcam

ab81551

Anti-Nucleophosmin (phospho T199) antibody [EP1857Y]

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.05% Sodium azide Constituents: 50% Tissue culture supernatant, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 0.1% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EP1857Y
Purification technique	Affinity purification Protein A
Concentration	0.089 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/2500 - 1/10000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100 - 1/250
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

IHC-P

Tested

Species	Human
Dilution info	1/100 - 1/250
Notes	-

Target data

See full target information NPM1 pT199

Function Involved in diverse cellular processes such as ribosome biogenesis, centrosome duplication, protein chaperoning, histone assembly, cell proliferation, and regulation of tumor suppressors p53/TP53 and ARF. Binds ribosome presumably to drive ribosome nuclear export. Associated with nucleolar ribonucleoprotein structures and bind single-stranded nucleic acids. Acts as a chaperonin for the core histones H3, H2B and H4. Stimulates APEX1 endonuclease activity on apurinic/apyrimidinic (AP) double-stranded DNA but inhibits APEX1 endonuclease activity on AP single-stranded RNA. May exert a control of APEX1 endonuclease activity within nucleoli devoted to repair AP on rDNA and the removal of oxidized rRNA molecules. In concert with BRCA2, regulates centrosome duplication. Regulates centriole duplication: phosphorylation by PLK2 is able to trigger centriole replication. Negatively regulates the activation of EIF2AK2/PKR and suppresses apoptosis through inhibition of EIF2AK2/PKR autophosphorylation.

Antagonizes the inhibitory effect of ATF5 on cell proliferation and relieves ATF5-induced G2/M blockade (PubMed:22528486). In complex with MYC enhances the transcription of MYC target genes (PubMed:25956029). May act as chaperonin or cotransporter in the nucleolar localization of transcription termination factor TTF1 (By similarity).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Storage information	Stable for 12 months at -20°C

Notes

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Nucleophosmin also known as NPM1 or B23 is a multifunctional nucleolar phosphoprotein with a molecular weight of approximately 37 kDa. It is highly expressed in the nucleolus of many human cells and is involved in various cellular processes. NPM1 aids ribosome biogenesis intracellular transport of ribosomal components and regulation of the centrosome. Its nucleocytoplasmic shuttling ability is due to its N-terminal domain which plays an important role in binding to

	nucleic acids and other proteins. Apart from these functions NPM1 acts as a histone chaperone and is important for maintaining genomic integrity.
Biological function summary	One of the central roles of NPM1 is its involvement in the regulation of cell growth and proliferation. NPM1 forms a complex with other nucleolar proteins affecting ribosome subunit assembly and transport. This protein also participates in the control of the ARF/p53 pathway by sequestering p53-regulating proteins which are essential for cell cycle control and apoptosis. Its interactions with proteins such as nucleolin and nucleophosmin-like proteins allow NPM1 to perform its functions in organizing the nucleolus and regulating responses to stress signals.
Pathways	The NPM1 protein is integrally involved in key cellular pathways like the regulation of the cell cycle and apoptosis. It directly interacts with pathways such as the ribosome biogenesis pathway and the p53 pathway. NPM1 has connections with proteins like ARF and MDM2 which play roles in retinoblastoma protein (pRb) regulation and in the control of tumor-suppressor activities. These pathways under NPM1's influence are critical for maintaining cellular homeostasis and ensuring proper cell cycle checkpoints.
Associated diseases and disorders	NPM1 mutations are commonly observed in acute myeloid leukemia (AML) where they affect the normal function of the protein leading to leukemogenesis. NPM1 is also linked to tumorigenesis through its impact on the p53 tumor suppressor pathway. Specifically alterations in NPM1 can disrupt the balance of other related proteins like ALK and p53 influencing the onset and progression of leukemia. Understanding the roles of NPM1 in these contexts is essential for developing targeted therapies to treat AML and other related disorders effectively.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

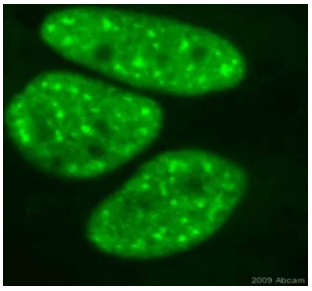
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

6 product images

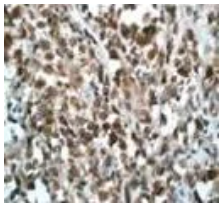


This image is courtesy of an anonymous customer review.

Immunocytochemistry/ Immunofluorescence - Anti-Nucleophosmin (phospho T199) antibody [EP1857Y] (ab81551)

Nucleophosmin (phospho T199) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Nucleophosmin (phospho T199) antibody

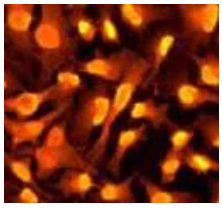
ab81551 staining Nucleophosmin (phospho T199) in human HeLa cells by Immunocytochemistry/ Immunofluorescence. Cells were fixed with formaldehyde, permeabilized with 0.1% Triton x100 in PBS and blocking with 5% serum was performed for 30 minutes at 23^0C. Samples were incubated with primary antibody (1/150 in PBS) for 1 hour at 23°C. An Alexa Fluor®488-conjugated goat polyclonal to rabbit IgG was used at dilution at 1/100 as secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nucleophosmin (phospho T199) antibody [EP1857Y] (ab81551)

Nucleophosmin (phospho T199) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Nucleophosmin (phospho T199) antibody

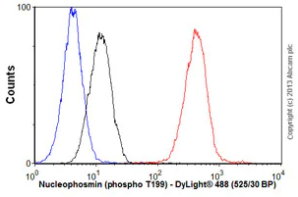
Immunohistochemistry analysis of paraffin-embedded Human lymphoma tissue, using 1/100 ab81551.
Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Nucleophosmin (phospho T199) antibody [EP1857Y] (ab81551)

Nucleophosmin (phospho T199) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Nucleophosmin (phospho T199) antibody

Immunofluorescent staing of HeLa cells using 1/100 ab81551.

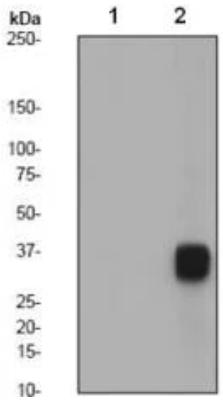


Flow Cytometry (Intracellular) - Anti-Nucleophosmin (phospho T199) antibody [EP1857Y] (ab81551)

Nucleophosmin (phospho T199) Flow Cytometry (Intracellular) staining using rabbit Anti-Nucleophosmin (phospho T199) antibody

Overlay histogram showing HeLa cells stained with ab76539 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab76539, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal)

(1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line). Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Western blot - Anti-Nucleophosmin (phospho T199) antibody [EP1857Y] (ab81551)

All lanes:
Western blot - Anti-Nucleophosmin (phospho T199) antibody [EP1857Y] (ab81551) at 1/2500 dilution

Lane 1:
lysate from untreated HeLa cells at 10 µg

Lane 2:
Lysate from HeLa cells serum-starved overnight, and then treated with 100ng/ml Calyculin A for 30min at 37☒ at 10 µg

Secondary

All lanes:
HRP labelled Goat anti-Rabbit at 1/2000 dilution

Predicted band size: 32 kDa

Observed band size: 33 kDa

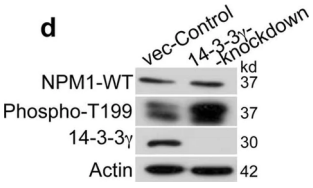


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Western blot - Anti-Nucleophosmin (phospho T199) antibody [EP1857Y] (ab81551)

Nucleophosmin (phospho T199) Western blot staining using rabbit Anti-Nucleophosmin (phospho T199) antibody

Nucleophosmin (phospho T199) western blot using anti-Nucleophosmin (phospho T199) antibody [EP1857Y] ab81551. Publication image and figure legend from Mukhopadhyay, A., Sehgal, L., et al., 2016, Sci Rep, PubMed 27253419.

ab81551 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab81551 please see the product overview.

Increased NPM1 phosphorylation at T199 leads to centrosome duplication in 14-3-3γknockdown cells.(a) Protein extracts from vector control or 14-3-3γ knockdown cells were resolved on SDS-PAGE followed by immuno-blot with the indicated antibodies. Actin served as loading control. (b,c) HCT116 cells were transfected with mCherry-α-tubulin and co-transfected with either the control plasmid (pCDNA3) or plasmids encoding wild type cdk1 (cdk1) or constitutively active cdk1 (cdk1AF). Post-transfection the cells were stained with antibodies to Cep170 (green) and DAPI (blue) (b). The percentage of mitotic cells with >2 centrosomes was determined in three independent experiments (c). (d) Protein extracts from the vector control and 14-3-3γ knockdown cells were resolved by SDS-PAGE, followed by Western blot with the indicated antibodies. Note that NPM1 is phosphorylated on T199 to a greater degree in the 14-3-3γ knockdown cells. Actin served as loading control. (e) The 14-3-3γ -knockdown and vector control cells were transfected with either the vector control or FLAG-epitope tagged versions of WT NPM1 or the NPM1 mutants (T199A and T199D). Post transfections, protein extracts prepared from these cells were resolved by SDS-PAGE followed by Western blot with the indicated antibodies. Western blots for actin serves as loading controls. (f,g) 14-3-3γ-knockdown and vector-control cells, transfected with mCherry-α-tubulin and FLAG-NPM1 constructs, were stained with antibodies to Cep-170, co-stained with DAPI

and followed by confocal microscopy (f) to determine the percentage of cells containing >2 centrosomes. The mean and standard deviation of three independent cells is plotted (g) p values were determined using a Student's t-test (2 sample unequal variance) with $p < 0.05$. Scale bars indicate 5 μm. All the Western blots were run under the same experimental conditions and the full length blots are in Supplementary Fig. 6.

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