abcam

Product datasheet

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



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Overview

Product name Anti-Nucleophosmin (phospho T199) antibody [EP1857Y]

Description Rabbit monoclonal [EP1857Y] to Nucleophosmin (phospho T199)

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, ICC/IF, Flow Cyt (Intra)

Species reactivity Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: lysate from HeLa cell treated with CA; IHC-P: Human lymphoma; ICC/IF: HeLa cells.

General notesThis 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 production

For more information see here.

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

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.

Storage buffer pH: 7.20

Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue

culture supernatant

Purity Protein A purified

Clonality Monoclonal

1

Clone number EP1857Y

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise quarantee covers the use of ab81551 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/2500 - 1/10000. Detects a band of approximately 33 kDa (predicted molecular weight: 33 kDa).
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF	★★★★ <u>(1)</u>	1/100 - 1/250.
Flow Cyt (Intra)		1/100. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

Target

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).

Involvement in disease

A chromosomal aberration involving NPM1 is found in a form of non-Hodgkin lymphoma. Translocation t(2;5)(p23;q35) with ALK. The resulting chimeric NPM1-ALK protein homodimerize and the kinase becomes constitutively activated.

A chromosomal aberration involving NPM1 is found in a form of acute promyelocytic leukemia. Translocation t(5;17)(q32;q11) with RARA.

A chromosomal aberration involving NPM1 is a cause of myelodysplastic syndrome (MDS). Translocation t(3;5)(q25.1;q34) with MLF1.

Defects in NPM1 are associated with acute myelogenous leukemia (AML). Mutations in exon 12 affecting the C-terminus of the protein are associated with an aberrant cytoplasmic location.

Sequence similarities

Belongs to the nucleoplasmin family.

Post-translational modifications

Acetylated at C-terminal lysine residues, thereby increasing affinity to histones. ADP-ribosylated.

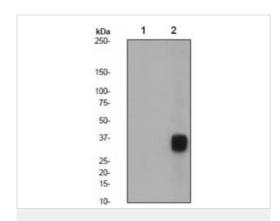
Phosphorylated at Ser-4 by PLK1 and PLK2. Phosphorylation at Ser-4 by PLK2 in S phase is required for centriole duplication and is sufficient to trigger centriole replication. Phosphorylation at Ser-4 by PLK1 takes place during mitosis. Phosphorylated by CDK2 at Ser-125 and Thr-199. Phosphorylation at Thr-199 may trigger initiation of centrosome duplication. Phosphorylated by CDK1 at Thr-199, Thr-219, Thr-234 and Thr-237 during cell mitosis. When these four sites are phosphorated, RNA-binding activity seem to be abolished. May be phosphorylated at Ser-70 by NEK2. The Thr-199 phosphorylated form has higher affinity for ROCK2. CDK6 triggers Thr-199 phosphorylation when complexed to Kaposi's sarcoma herpesvirus (KSHV) V-cyclin, leading to viral reactivation by reducing viral LANA levels.

Sumoylated by ARF.

Cellular localization

Nucleus, nucleolus. Nucleus, nucleoplasm. Cytoplasm, cytoskeleton, microtubule organizing center, centrosome. Generally nucleolar, but is translocated to the nucleoplasm in case of serum starvation or treatment with anticancer drugs. Has been found in the cytoplasm in patients with primary acute myelogenous leukemia (AML), but not with secondary AML. Can shuttle between cytoplasm and nucleus. Co-localizes with the methylated form of RPS10 in the granular component (GC) region of the nucleolus. Colocalized with nucleolin and APEX1 in nucleoli. Isoform 1 of NEK2 is required for its localization to the centrosome during mitosis.

Images



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

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

Lane 1: lysate from untreated HeLa cells

Lane 2: lysate from HeLa cells treated with CA

Lysates/proteins at 10 µg per lane.

Secondary

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

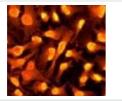
Predicted band size: 33 kDa **Observed band size:** 33 kDa



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

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.



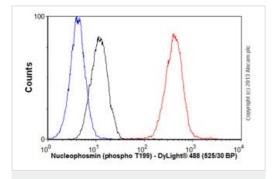
Immunocytochemistry/ Immunofluorescence - Anti-Nucleophosmin (phospho T199) antibody [EP1857Y] (ab81551) Immunofluorescent staing of HeLa cells using 1/100 ab81551.



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

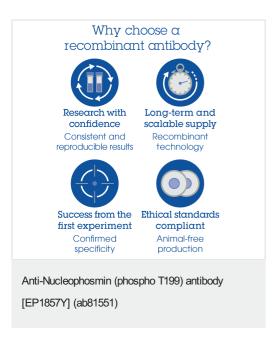
This image is a courtesy of Anonymous Abreview

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°C. 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.



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

Overlay histogram showing HeLa cells stained with <u>ab76539</u> (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 (<u>ab76539</u>, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit lgG (H+L) (<u>ab96899</u>) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1µg/1x10⁶ 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.



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