abcam

Product datasheet

Anti-Nucleophosmin (phospho T199) antibody ab59353

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Overview

Product name	Anti-Nucleophosmin (phospho T199) antibody
Description	Rabbit polyclonal to Nucleophosmin (phospho T199)
Host species	Rabbit
Specificity	ab59353 detects endogenous levels of Nucleophosmin only when phosphorylated at threonine 199. Human: Thr199; Mouse: Thr198; Rat: Thr198.
Tested applications	**Suitable for:** ICC/IF, WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide corresponding to Human Nucleophosmin. Synthesized phosphopeptide derived from human Nucleophosmin around the phosphorylation site of threonine 199 (RDTPPA). Database link: P06748
General notes	Store at -20°C for 1 year

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine), 0.87% Sodium chloride Without Mg+2 and Ca+2
Purity	Immunogen affinity purified
Purification notes	Affinity purified from rabbit antiserum by affinity chromatography using epitope specific phosphopeptide. The antibody against non phosphopeptide was removed by chromatography using non phosphopeptide corresponding to the phosphorylation site.
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab59353** 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
ICC/IF		Use a concentration of 1 - 5 µg/ml.
WB	★★★★★	1/500 - 1/1000. Detects a band of approximately 33 kDa.
IHC-P		1/50 - 1/100.

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

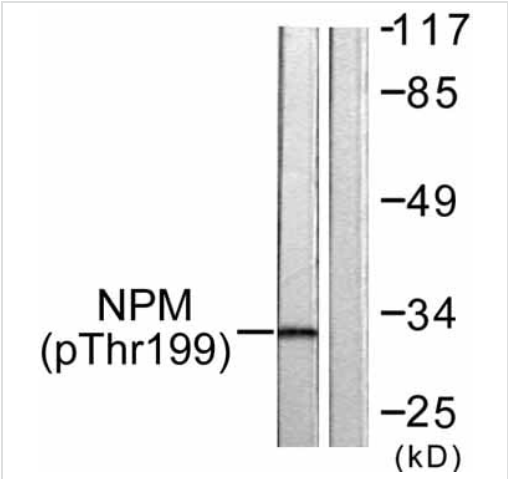
Acetylated at C-terminal lysine residues, thereby increasing affinity to histones.
ADP-ribosylated.
Phosphorylated at Ser-4 by PLK1 and PLK2. Phosphorylation at Ser-4 by PLK2 in S phase is required for centriole duplication and is sufficient to trigger centriole replication. Phosphorylation at Ser-4 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

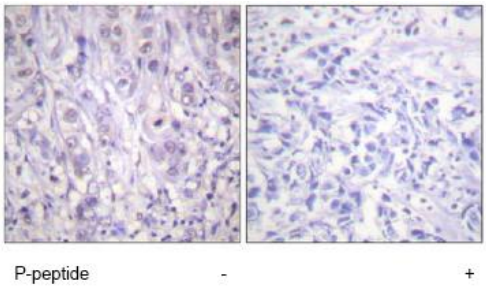


Western blot - Anti-Nucleophosmin (phospho T199) antibody (ab59353)

All lanes : Anti-Nucleophosmin (phospho T199) antibody (ab59353) at 1/500 dilution

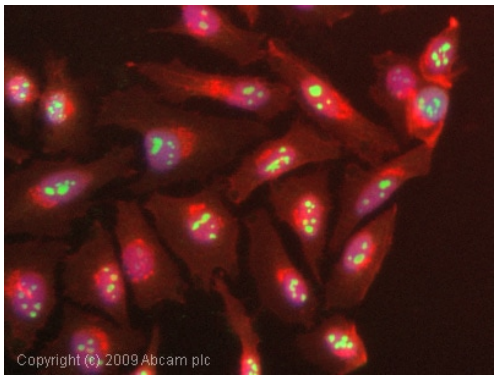
Lane 1 : Extracts from Jurkat cells with no immunizing peptide
Lane 2 : Extracts from Jurkat cells with immunizing peptide

Observed band size: 33 kDa
why is the actual band size different from the predicted?



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nucleophosmin (phospho T199) antibody (ab59353)

ab59353, at a 1/50 dilution, staining human Nucleophosmin in breast carcinoma, using Immunohistochemistry, Paraffin embedded tissue, in the absence (left image) and in the presence of the immunizing peptide (right image).



Immunocytochemistry/ Immunofluorescence - Anti-Nucleophosmin (phospho T199) antibody (ab59353)

ICC/IF image of ab59353 stained HeLa cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab59353, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

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