abcam

Product datasheet

Anti-Nucleophosmin antibody [5E3] ab40696

3 References 4 Images

Overview

Product name	Anti-Nucleophosmin antibody [5E3]
Description	Mouse monoclonal [5E3] to Nucleophosmin
Host species	Mouse
Tested applications	**Suitable for:** ICC/IF, Flow Cyt, ELISA, WB, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment, corresponding to amino acids 81-294 of Human Nucleophosmin
Positive control	This antibody gave a positive result when used in the following methanol fixed cell lines: HCT116

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term.
Storage buffer	Preservative: 0.1% Sodium Azide Constituents: PBS, pH 7.4
Purity	Protein G purified
Clonality	Monoclonal
Clone number	5E3
Myeloma	Sp2/0
Isotype	IgG2b
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab40696 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 10 µg/ml.

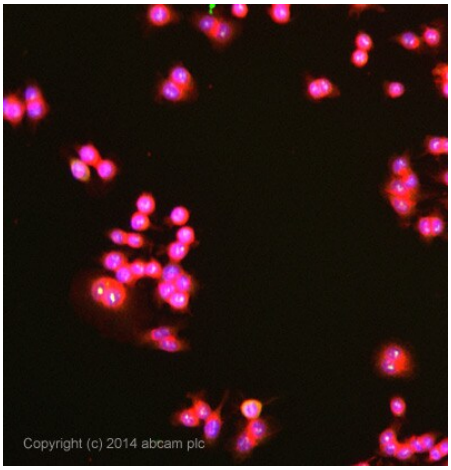
Application	Abreviews	Notes
Flow Cyt		Use 2-5µg for 10^6 cells.
ELISA		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 33 kDa.
IHC-P		Use at an assay dependent concentration.

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

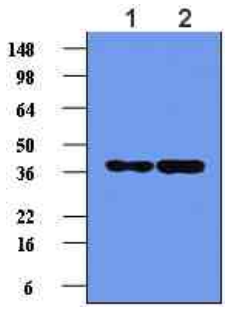
center, centrosome. Generally nucleolar, but is translocated to the nucleoplasm in case of serum starvation or treatment with anticancer drugs. Has been found in the cytoplasm in patients with primary acute myelogenous leukemia (AML), but not with secondary AML. Can shuttle between cytoplasm and nucleus. 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



Immunocytochemistry/ Immunofluorescence - Anti-Nucleophosmin antibody [5E3] (ab40696)

ICC/IF image of ab40696 stained HCT116 cells. The cells were 100% methanol fixed (5 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 ab40696 at 10µg/ml overnight at +4°C. The secondary antibody (pseudo-colored green) was Alexa Fluor® 488 goat anti- mouse (**ab150117**) IgG (H+L) preadsorbed, used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1h at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1hour at room temperature.



Western blot - Anti-Nucleophosmin antibody [5E3] (ab40696)

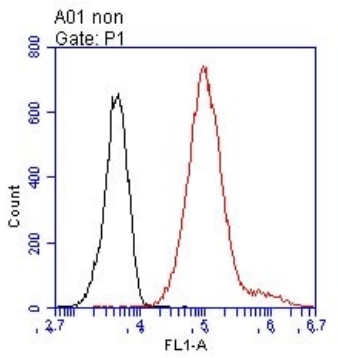
All lanes : Anti-Nucleophosmin antibody [5E3] (ab40696) at 1/1000 dilution

Lane 1 : HeLa cell lysate
Lane 2 : HepG2 cell lysate

Lysates/proteins at 5 µg per lane.

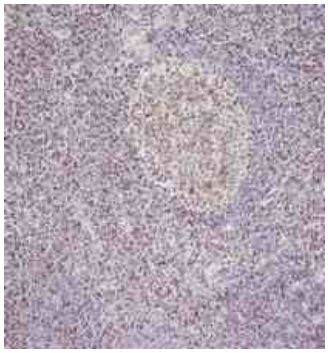
Predicted band size: 33 kDa
Observed band size: 37 kDa

Proteins were visualised using a goat anti-mouse secondary antibody conjugated to HRP and an ECL detection system.



Flow Cytometry - Anti-Nucleophosmin antibody [5E3] (ab40696)

Flow cytometry analysis of Nucleophosmin in HeLa cells, using ab40696 2-5 µg for 1x10^6 cells (red line). The secondary antibody used was an Alexa Fluor® 488 conjugated goat anti-mouse IgG. The isotype control antibody was mouse IgG (black line).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nucleophosmin antibody [5E3] (ab40696)

Ab40696 (1:100) staining human Nucleophosmin in human palatine tonsil tissue by immunohitochemistry using paraffin embedded tissue.

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