Datasheet for ABIN1737584

anti-NPM1 antibody (C-Term)

3 Validations

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Overview

Quantity:	100 µL
Target:	NPM1
Binding Specificity:	AA 250-298, C-Term
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Application:	Western Blotting (WB), Immunofluorescence (IF), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p)), Immunoprecipitation (IP), Flow Cytometry (FACS)

Product Details

Immunogen:	Synthetic peptide made to a C-terminal region of human mutant nucleophosmin (within residues 250-294) [AAW67752]
Specificity:	Reacts with the mutant form of Nucleophosmin
Purification:	Antigen affinity purified

Target Details

Target:	NPM1
Alternative Name:	Nucleophosmin (NPM1 Products)
Background:	Nucleophosmin (NPM) is a multi-functional protein that is involved in many cellular activities including cell growth, proliferation and transformation. It is frequently overexpressed in solid tumors, and has recently been found to be mutated and aberrantly localized in the cytoplasm of

Target Details

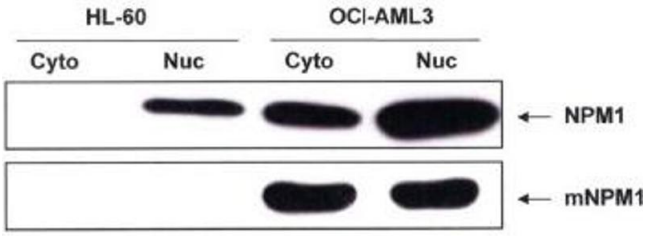
	leukaemic blasts in patients with acute myeloid leukemia (AML).
Gene ID:	4869
Pathways:	Ribonucleoprotein Complex Subunit Organization, Ribosome Assembly

Application Details

Application Notes:	Working dilution: Optimal dilution should be determined by the end user. The following are guidelines only : FC(1:100) ICC/IF(1:50 - 1:100) IHC - P(1:50 - 1:100) IP(1:50) WB(1:1000)
Restrictions:	For Research Use only

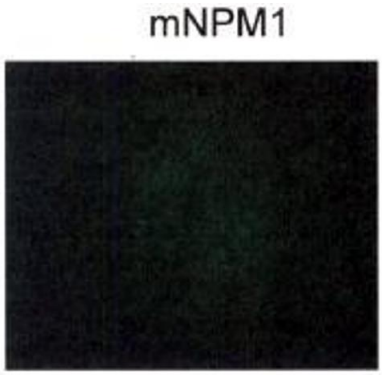
Handling

Format:	Liquid
Concentration:	1 mg/mL
Buffer:	PBS pH 7.4, 0.02 % sodium azide
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C/-20 °C
Storage Comment:	Short term storage at +4°C. For extended periods store in aliquots at -20°C. Antibodies are guaranteed for 6 month from date of receipt.
Expiry Date:	6 months



Detection of mutant nucleophosmin in OCI-AML3 lysates using pab50321

Image 1.



NPM1 localization by immunofluorescence in HL-60 cells (negative control) using pab50321

Image 2.

Successfully validated (Western Blotting (WB))

by Institute of Hematology and Blood Transfusion, Prague

Report Number: 101096

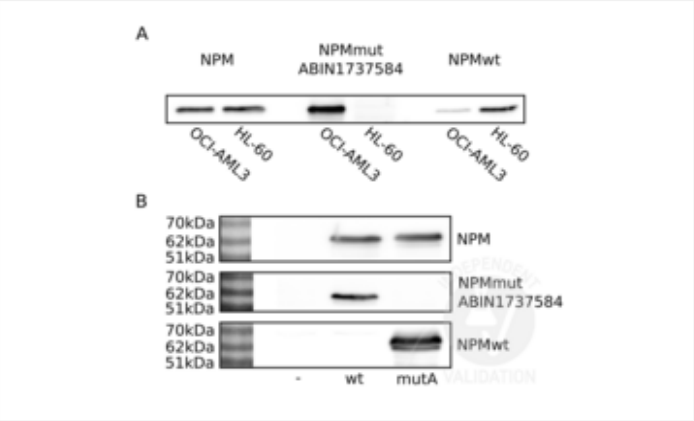
Date: May 17 2017

Target:	NPM1
Method validated:	Western Blotting (WB)
Positive Control:	OCI-AML3 cells containing the NPM1 mutation type A, HEK-293T transfected with GFP-NPM mutA
Negative Control:	HL-60 cells
Notes:	Passed. ABIN1737584 specifically recognizes mutated nucleophosmin in OCI-AML3 cells and NPMmut transfected HEK-293T cells.
Primary Antibody:	ABIN1737584
Secondary Antibody:	goat-anti rabbit antibody (LifeTechnologies, cat. No.31460)
Protocol:	• Grow OCI-AML3 (DSMZ) cells in MEM medium (Sigma-Aldrich, M8042, lot RNBF-0384) supplemented with 20% FBS (Biochrom, S0615, lot 0513C) and penicillin-streptomycin (Sigma-Aldrich, P4333, lot 066M4791V) at 37°C and 5% CO_2 in 5ml on a tissue culture dish to $1.5x10^6$ cells/ml. • Grow HL-60 (ECACC) cells RPMI medium (Sigma-Aldrich, R0883, lot RNBF-7081) supplemented with 10% FBS and penicillin-streptomycin at 37°C and 5% CO_2 in 5ml on a tissue culture dish to $1.5x10^6$ cells/ml. • Grow HEK-293T cells on DMEM medium (Sigma-Aldrich, D8062, lot RNBF-4838) supplemented with 10% FBS and penicillin-streptomycin at 37°C and 5% CO_2 in 5ml on a tissue culture dish to 60% confluency. • Transfect HEK-293T cells with plasmid containing gene for GFP-NPMwt or GFP-NPMmutA using jetPRIME transfection reagent (Polyplus Transfection, 114-15, lot 1240106I17) following the manufacturer´s instructions. • Collect 3-5x106 cells directly in 100µl 1x Laemmli SDS sample buffer, and boil them for 5min at 95°C. • Centrifuge samples at 200.000g/4°C for 4h. Take of the supernatant and store at -20°C. • Separate 10µl of each sample on a freshly cast Tris HCl pH8.8 12,5% SDS-PAGE in a Mini-PROTEAN Tetra Cell System for 1h at 200V/4°C. • Transfer proteins onto PVDF membrane (GE Healthcare, 10600023, lot A10125999) with a semi-dry blotting system for 1.5h at 100mA. • Block the membrane with 5% non-fat dry milk in PBS-Tween (0.5% v/v) for 1h at RT.

- Incubation the membrane with primary
  - rabbit anti-mutant nucleophosmin (antibodies-online, ABIN1737584) diluted 1:1000 in 5% non-fat dry milk in PBSTON at 4°C,
  - mouse anti-NPM (Santa Cruz, sc-70392) recognizing the N-terminus of NPM protein (i.e. both the NPM wild-type and NPM mutant type A) diluted 1:500 in 5% non-fat dry milk in PBSTON at 4°C, or
  - mouse anti-NPM (Santa Cruz, sc-271737) recognizing the C-terminus of NPM wild-type protein diluted 1:500 in 5% non-fat dry milk in PBSTON at 4°C.
- Wash the membrane 6x for 5min in PBST.
- Incubate the membrane with secondary HRP-conjugated
  - goat-anti rabbit antibody (LifeTechnologies, cat. No.31460) diluted 1:50.000 in PBST for 1h at RT or
  - goat-anti mouse antibody (LifeTechnologies, cat. No.31430) diluted 1:50.000 in PBST for 1h at RT.
- Wash membrane 6x for 5 min with PBS-Tween.
- Visualize protein bands using ECL Plus detection reagent (GE Healthcare, RPN2232, lot 9732255) on a G-box iChemi XT4 digital imaging device (Syngene Europe).

Experimental Notes: The nucleophosmin antibody ABIN1737584 reveals a protein of the expected molecular weight in lysates of OCI-AML3 and of HEK-293T transfected with GFP-NPM mutA. The protein band is but not visible in the HL-60 negative control. A weak band from OCI-AML3 detected by an anti-NPMwt antibody confirms the concurrent presence of both NPMmutA and NPMwt in the OCI-AML3 cell line.

Image for Validation report #101096



Validation image no. 1 for anti-Nucleophosmin (Nucleolar phosphoprotein B23, Numatrin) (NPM1) (AA 250-298), (C-Term) antibody (ABIN1737584)

A. Detection of the nucleophosmin N-terminus (NPM), NPM mutation type A C-terminus (NPMmut), or C-terminus of NPM wb (NPMwt) by three different antibodies in OCI-AML3 and HL-60 cell lysates as described in the protocol. B. Nucleophosmin detection in lysates from untransfected HEK-293T cells (-), cells transfected with GFP-NPMwt (wt), or GFP-NPMmutA (mutA) with antibodies recognizing the N-terminus (NPM), the C-terminus of NPM mutation type A (NPMmut), or the C-terminus of wt nucleophosmin

(NPMwt).

Successfully validated (Immunofluorescence (IF))

by Institute of Hematology and Blood Transfusion, Prague

Report Number: 101288

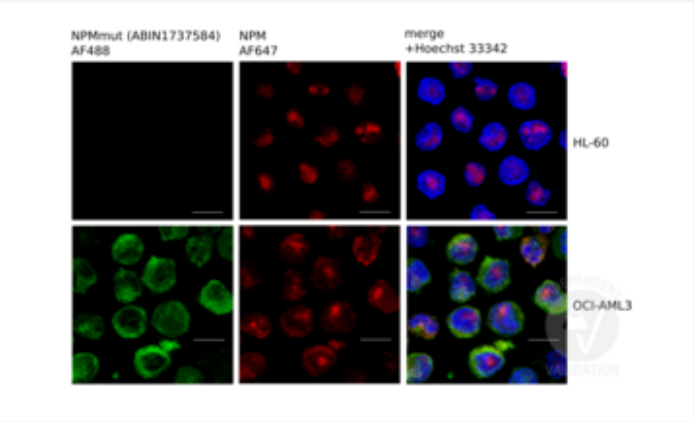
Date: May 17 2017

Target:	NPM1
Method validated:	Immunofluorescence (IF)
Positive Control:	OCI-AML3 cells containing the NPM1 mutation type A
Negative Control:	HL-60 cells
Notes:	Passed. ABIN1737584 specifically recognizes mutated nucleophosmin in sample.
Primary Antibody:	ABIN1737584
Secondary Antibody:	AlexaFluor488-conjugated goat anti-rabbit antibody (LifeTechnologies, A31627, lot 1440410)
Protocol:	• Grow OCI-AML3 (DSMZ) cells in MEM medium (Sigma-Aldrich, M8042, lot RNBF-0384) supplemented with 20% FBS (Biochrom, lot 0513C) and penicillin-streptomycin (Sigma-Aldrich, P4333, lot 066M4791V) at 37°C and 5% CO_2 in 5ml on a tissue culture dish to $1.5x10^6$ cells/ml. • Grow HL-60 (ECACC) cells RPMI medium (Sigma-Aldrich, R0883, lot RNBF-7081) supplemented with 10% FBS and penicillin-streptomycin at 37°C and 5% CO_2 in 5ml on a tissue culture dish to $1.5x10^6$ cells/ml. • Wash 10^6 cells with PBS, resuspend them in 150µl PBS and apply the suspension on a coverslip in a humidified chamber for 20min. • Aspirate PBS with thick filter paper. • Apply 4% paraformaldehyde for 1h at RT. • Wash the coverslip briefly with PBS and apply 0.2% Triton X-100 for 10min at RT. • Wash the coverslip 3x for 5 min with PBS-Tween (PBS, Tween 20 0.2% v/v). • Incubate the coverslip with image iT-signal enhancer (LifeTechnologies, from A31627 kit) for 30min at RT. • Wash the coverslip 3x for 5min with PBST. • Incubate the coverslip with primary rabbit anti-mutant nucleophosmin(antibodies-online, ABIN1737584) diluted 1:100 and mouse monoclonal anti-NPM (Santa Cruz, sc-70392) diluted 1:50 in PBST for 1h at RT. • Wash the coverslip 3x for 5 min with PBST. • Incubation the coverslip with secondary AlexaFluor488-conjugated goat anti-rabbit antibody (LifeTechnologies, A31627, lot 1440410), AlexaFluor647-conjugated goat anti-mouse antibody (LifeTechnologies, A31625, lot 1626792) diluted 1:200 and 1µM Hoechst33342 in PBST for 1h at RT in the dark.

- Wash the membrane 2x for 5min with PBST.
- Wash the membranc for 5 min in PBS.
- Apply 10µl of ProLong Gold Antifade Mountant (LifeTechnologies, P36930) on a microscope slide and attach it to a coverslip. Dry ON.
- Visualize the fluorescence signal under confocal laser scanning microscope FluoView FV1000 (Olympus Corporation) at 60x/1.35 oil objective.

Experimental Notes:	The nucleophosmin antibody ABIN1737584 reveals a protein of the expected (cytoplasmic) localization of antigen in OCI-AML3 cell line. The protein is only visible in the positive but not the negative control (HL-60).

Image for Validation report #101288



Validation image no. 1 for anti-Nucleophosmin (Nucleolar phosphoprotein B23, Numatrin) (NPM1) (AA 250-298), (C-Term) antibody (ABIN1737584)

Staining of PFA fixed OCI-AML3 and HL-60 cells with ABIN1737584 recognizing the C-terminus of NPM mutation type A (green) and a different antibody specific for the NPM N-terminus (red). Counterstaining of the nuclei with Hoechst33342 (blue). Bars represent 10µm.

Validation report #101289 for Flow Cytometry (FACS)

Successfully validated (Flow Cytometry (FACS))

by Institute of Hematology and Blood Transfusion, Prague

Report Number: 101289

Date: May 17 2017

Target:	NPM1
Method validated:	Flow Cytometry (FACS)
Positive Control:	OCI-AML3 cells containing the NPM1 mutation type A
Negative Control:	HL-60 cells
Notes:	Passed. ABIN1737584 specifically recognizes mutated nucleophosmin in OCI-AML3 cells.
Primary Antibody:	ABIN1737584
Secondary Antibody:	AlexaFluor488-conjugated goat anti-rabbit antibody (LifeTechnologies, A31627, lot 1440410)
Isotype:	normal rabbit IgG (Santa Cruz, sc-2027)

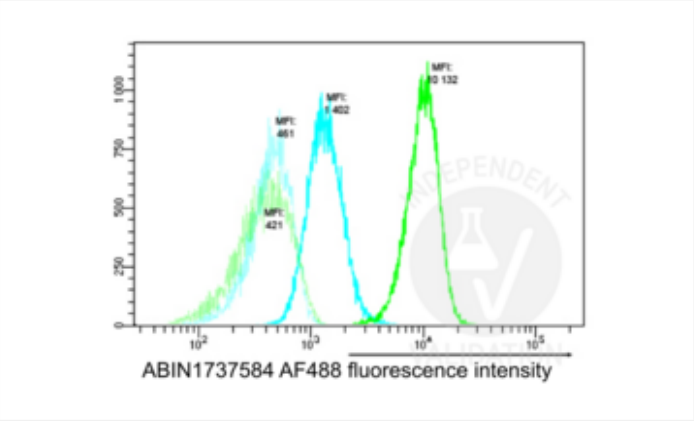
Protocol:

- Grow OCI-AML3 (DSMZ) cells in MEM medium (Sigma-Aldrich, M8042, lot RNBF-0384) supplemented with 20% FBS (Biochrom, S0615, lot 0513C) and penicillin-streptomycin (Sigma-Aldrich, P4333, lot 066M4791V) at 37°C and 5% CO_2 in 5ml on a tissue culture dish $1.5x10^6$ cells/ml.
- Grow HL-60 (ECACC) cells RPMI medium (Sigma-Aldrich, R0883, lot RNBF-7081) supplemented with 10% FBS and penicillin-streptomycin at 37°C and 5% CO_2 in 5ml on a tissue culture dish $1.5x10^6$ cells/ml.
- Wash $5x10^5$ cells with PBS, resuspend them in 200µl of 4% paraformaldehyde.
- Incubate for 15min at RT.
- Centrifuge 5min/500g/RT, aspirate the supernatant.
- Resuspend cells in 50µl of ice-cold PBS and add 450µl of ice-cold methanol.
- Store resuspended cells at -20°C for at least 30min (max 30 days).
- Wash the cells 2x with PBS-BSA (1% BSA in PBS) and centrifuge them for 5min/500g.
- Resuspend cells in 200µl PBS-BSA.
- Incubate cells for 20min at RT.
- Incubate cells with primary antibody
  - rabbit anti-mutant nucleophosmin(antibodies-online, ABIN1737584) diluted 1:100 in PBS-BSA for 1h at RT or
  - normal rabbit IgG (Santa Cruz, sc-2027) as negative staining control diluted 1:100 in PBS-BSA for 1h at RT.
- Wash the cells 2x with PBS-BSA (1% BSA in PBS) and centrifuge them for 5min/500g.
- Resuspend cells in 200µl PBS-BSA.

- Incubate cells with secondary AlexaFluor488-conjugated goat anti-rabbit antibody (LifeTechnologies, A31627, lot 1440410) diluted 1:200 in PBS-BSA for 1h at RT.
- Wash the cells 2x with PBS-BSA (1% BSA in PBS) and centrifuge them for 5min/500g.
- Resuspend pellet in 300µl PBS and acquire AlexaFluor488 signal on flow-cytometer (BD Fortessa).

Experimental Notes: The nucleophosmin antibody ABIN1737584 reveals a strong fluorescence signal from OCI-AML3 cell line. Only moderate signal is obtained from the negative control (HL-60) and no signal is detected with non-specific primary antibody (normal rabbit IgG).

Image for Validation report #101289



Validation image no. 1 for anti-Nucleophosmin (Nucleolar phosphoprotein B23, Numatrin) (NPM1) (AA 250-298), (C-Term) antibody (ABIN1737584)

OCI-AML3 (green lines) and HL-60 (cyan lines) cells were stained with antibody recognizing C-terminus of NPM mutation type A (ABIN1737584, full lines) or normal rabbit IgG (dotted lines) as described in the protocol.