abcam

Product datasheet

Anti-Fibrillarin antibody [38F3] - Nucleolar Marker ab4566

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Overview

Product name Anti-Fibrillarin antibody [38F3] - Nucleolar Marker

Description Mouse monoclonal [38F3] to Fibrillarin - Nucleolar Marker

Host species Mouse

Specificity This clone was selected because it stains a single ~34kDa band on western blotting and shows a

clear and strong punctate staining of yeast nuclei. It can therefore be used to identify nucleoli immunocytochemically. ab4566 was raised against yeast nuclear preps and the immunogen was identified as Nop1p, the yeast homolog of fibrillarin. Due to high aa homology the antibody should

work with any specie possessing a nucleus, however this has not been tested.

Tested applications Suitable for: Flow Cyt, ICC/IF, WB

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Plants 4

Immunogen Tissue, cells or virus corresponding to Saccharomyces cerevisiae Fibrillarin.

Yeast nuclear preparation (S. cerevisiae). Hybridomas were screened by immunofluorescence on

yeast cells and by western blotting on yeast protein homogenates (S. cerevisiae).

Positive control ICC: HEK-293 cells and SH-SY5Y cells; WB: HEK-293, C6, NIH-3T3 nuclear fractions

General notes Pfam number: PF01269. A reference below describes the characterization of D77, an antibody

very similar but not identical to ab4566.

Gives a much weaker signal in western blot compared to <u>ab218846</u>.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

Properties

Form Liquid

1

Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw Storage instructions

cycles.

Storage buffer Preservative: 0.065% Sodium azide

Constituent: Tissue culture supernatant

Purity Tissue culture supernatant

Purification notes Sterile filtered. Clonality Monoclonal

Clone number 38F3 Isotype lgG1

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab4566 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
Flow Cyt		1/20. ab170190 - Mouse monoclonal lgG1, is suitable for use as an isotype control with this antibody.
ICC/IF	****(9)	1/100. For IF of mammalian cells 1/500. ab4566 is sensitive to aldehyde. Use a mild formalin fixation or acetone or methanol fixation as the target is nuclear.
WB	★★★★★ (10)	1/2000 - 1/10000. Detects a band of approximately 34 kDa. 1/2000 (cell lysates) - 1/10000 (nuclear fractions)(ECL). For other (non-ECL) western detection methods, 1/1000 - 1/5000. To detect mammalian fibrillarin on western blots by ECL, 1/500.

Target

Function S-adenosyl-L-methionine-dependent methyltransferase that has the ability to methylate both RNAs

and proteins. Involved in pre-rRNA processing by catalyzing the site-specific 2'-hydroxyl

methylation of ribose moieties in pre-ribosomal RNA. Site specificity is provided by a guide RNA that base pairs with the substrate. Methylation occurs at a characteristic distance from the sequence involved in base pairing with the guide RNA. Also acts as a protein methyltransferase

by mediating methylation of 'Gln-105' of histone H2A (H2AQ104me), a modification that impairs binding of the FACT complex and is specifically present at 35S ribosomal DNA locus

(PubMed:24352239).

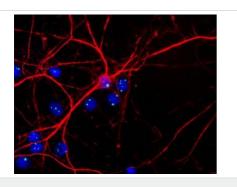
Sequence similarities Belongs to the methyltransferase superfamily. Fibrillarin family.

Post-translational

By homology to other fibrillarins, some or all of the N-terminal domain arginines are modified to modifications asymmetric dimethylarginine (DMA).

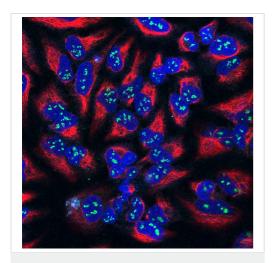
Cellular localization Nucleus, nucleolus. Fibrillar region of the nucleolus.

2



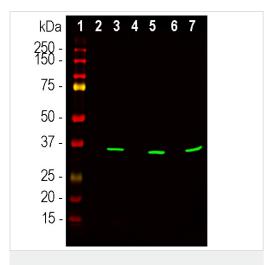
Immunocytochemistry/ Immunofluorescence - Anti-Fibrillarin antibody [38F3] - Nucleolar Marker (ab4566)

Rat neurons and glial stained with mouse monoclonal to Fibrillarin (green) and with chicken antibody to neurofilament NF-H (red). Cells were counterstained with a fluorescent DNA probe (blue). Nuclear DNA is revealed with Hoechst dye (blue). Cultures were processed using our standard fixation and staining procedure (in protocol section).



Immunocytochemistry/ Immunofluorescence - Anti-Fibrillarin antibody [38F3] - Nucleolar Marker (ab4566)

ICC analysis of HeLa cells stained with mouse monoclonal to Fibrillarin (green) and with chicken antibody to vimentin (red) and counterstained with a fluorescent DNA probe (blue). Nuclear DNA is revealed with DAPI(blue). The vimentin antibody was used at a dilution of 1/1000 and the fibrillarin monoclonal at 1/100. Cultures were processed using standard fixation and staining procedure (in protocol section).



Western blot - Anti-Fibrillarin antibody [38F3] - Nucleolar Marker (ab4566)

All lanes : Anti-Fibrillarin antibody [38F3] - Nucleolar Marker (ab4566) at 1/500 dilution

Lane 2: C6 cytosol fraction

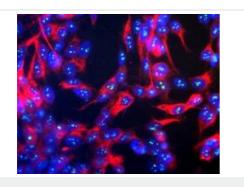
Lane 3: C6 nuclear fraction

Lane 4: HEK-293 cytosol fraction

Lane 5: HEK-293 nuclear fraction **Lane 6**: NIH-3T3 cytosol fraction

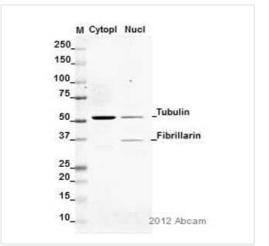
Lane 7: NIH-3T3 nuclear fraction

Observed band size: 37 kDa



Immunocytochemistry/ Immunofluorescence - Anti-Fibrillarin antibody [38F3] - Nucleolar Marker (ab4566)

Human neuroblastoma line SH-SY5Y stained with mouse monoclonal to Fibrillarin (green) and with chicken antibody to neurofilament NF-H (red) and counterstained with a fluorescent DNA probe (blue). Nuclear DNA is revealed with Hoechst dye (blue). The NF-H antibody was used at a dilution of 1/100000 and the fibrillarin monoclonal at 1/1000. Cultures were processed using standard fixation and staining procedure (in protocol section).



Western blot - Anti-Fibrillarin antibody [38F3] - Nucleolar Marker (ab4566) Image courtesy of an anonymous Abreview.

Mouse embryonic fibroblast fractionation.

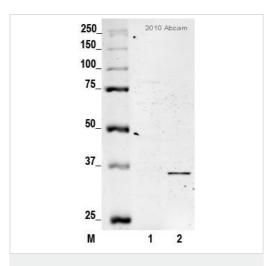
Cytopl - cytoplasmic fraction.

Nucl - nuclear fraction.

20 µg of each loaded.

ab4566 used at a 1/2000 dilution.

The secondary used was an Alexa-Fluor 680 conjugated goat antimouse polyclonal used at a 1/10000 dilution.



Western blot - Anti-Fibrillarin antibody [38F3] -Nucleolar Marker (ab4566)

Image is courtesy of Dr Svetlana Khoronenkova

All lanes : Anti-Fibrillarin antibody [38F3] - Nucleolar Marker (ab4566) at 1/2000 dilution (incubated for 1 hour, diluted with Blocking buffer 1:1 in PBS+0.1%Tween)

Lane 1 : cytoplasmic protein fraction of HeLa cells with LFCOR® Odyssey® Blocking Buffer, 45 minutes at room temperature at 50 %

Lane 2: nuclear protein fraction of HeLa cells with LI-COR®Odyssey® Blocking Buffer, 45 minutes at room temperature at 50 %

Lysates/proteins at 20 µg per lane.

Secondary

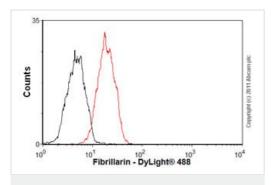
All lanes: AlexaFluor 680 goat anti-mouse at 1/10000 dilution

Performed under reducing conditions.

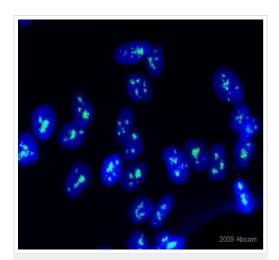
Additional bands at: 34 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 1 second

Overlay histogram showing HEK293 cells stained with ab4566 (red line). The cells were fixed with 80% methanol (5 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 (ab4566, 1/20 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse lgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse lgG1 [ICIGG1](ab91353, 2µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed.



Flow Cytometry - Anti-Fibrillarin antibody [38F3] - Nucleolar Marker (ab4566)



Immunocytochemistry/ Immunofluorescence - Anti-Fibrillarin antibody [38F3] - Nucleolar Marker (ab4566)

Image is courtesy of Cesar Camacho

ab staining Fibrillarin in Human melanoma A7 cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with 0.1% Triton and blocked with 1% BSA for 1 hour at room temperature. Samples were incubated with primary antibody (1/500 in 1% BSA) for 24 hours at 4°C. A FITC-conjugated Goat anti-mouse polyclonal (1/200) was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Fibrillarin antibody [38F3] - Nucleolar Marker (ab4566)

High magnification view of human Hek293 cell nuclei stained with mouse monoclonal to fibrillarin (green), counterstained with a fluorescent DNA probe (blue). Nuclear DNA is revealed with Hoechst dye (blue). Cultures were processed using our standard fixation and staining procedure (in protocol section).

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