Datasheet for ABIN968450

anti-NONO antibody (AA 368-471)

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Overview

Quantity:	50 µg
Target:	NONO
Binding Specificity:	AA 368-471
Reactivity:	Human, Mouse, Rat, Dog
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This NONO antibody is un-conjugated
Application:	Western Blotting (WB), BioImaging (BI)

Product Details

Immunogen:	Human p54 [nrb] aa. 368-471
Clone:	3-p54nrb
Isotype:	IgG1
Cross-Reactivity:	Dog (Canine), Mouse (Murine), Rat (Rattus)
Characteristics:	1. Since applications vary, each investigator should titrate the reagent to obtain optimal results. 2. Please refer to us for technical protocols. 3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance. 4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive

Product Details

	deposits in plumbing. 5. Source of all serum proteins is from USDA inspected abattoirs located in the United States. 6. Triton is a trademark of the Dow Chemical Company.
Purification:	The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Target Details

Target:	NONO
Alternative Name:	p54 nrb (NONO Products)
Background:	Splicing, the removal of introns from pre-mRNA, is mediated by spliceosomal complexes that contain multiple snRNPs and a number of non-snRNP splicing factors, such as hPRP17 and hPRP18. p54 [nrb] (nuclear RNA-binding protein, 54 kDa) is a non-snRNP RNA splicing factor. It binds and enhances the activity of topoisomerase I, the enzyme that nicks DNA to relieve torsional stress during replication and transcription. p54 [nrb] is the the human homolog of mouse NonO and bovine IPEB. The N-terminal half of p54 [nrb] contains two RNA recognition motifs (RRMs), that allow it to bind single stranded RNA. These motifs are included in a region that is 71% homologous with the human splicing factor PSF and 42% homologous with the Drosophila puff-specific protein BJ6. In addition to its role in RNA splicing, p54 [nrb] functions as a DNA transcription factor in multiple cell types. The N-terminal portion of p54 [nrb] also mediates its interaction with DNA. The ability of p54 [nrb] to bind DNA, but not RNA, is thought to require post-translational modification. Thus, p54 [nrb] can function as either an RNA splicing factor or a DNA transcription factor in a post-translational-dependent manner.
Molecular Weight:	60 kDa

Application Details

Application Notes:	Bioimaging 1. Seed the cells in appropriate culture medium at ~10,000 cells per well in an 96-well Imaging Plate and culture overnight. 2. Remove the culture medium from the wells, and fix the cells by adding 100 myl of Fixation Buffer to each well. Incubate for 10 minutes at room temperature (RT). 3. Remove the fixative from the wells, and permeabilize the cells using either 90% methanol, or Triton™ X-100: a. Add 100 myl of -20°C 90% methanol to each well and incubate for 5 minutes at RT. OR b. Add 100 myl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.

Application Details

	4. Remove the permeabilization buffer, and wash the wells twice with 100 myl of 1× PBS. 5. Remove the PBS, and block the cells by adding 100 myl of to each well. Incubate for 30 minutes at RT. 6. Remove the blocking buffer and add 50 myl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT. 7. Remove the primary antibody, and wash the wells three times with 100 myl of 1× PBS. 8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 myl to each well, and incubate in the dark for 1 hour at RT. 9. Remove the second step reagent, and wash the wells three times with 100 myl of 1× PBS. 10. Remove the PBS, and counter-stain the nuclei by adding 200 myl per well of 2 myg/ml Hoechst 33342 in 1× PBS to each well at least 15 minutes before imaging. 11. View and analyze the cells on an appropriate imaging instrument.
Comment:	Related Products: ABIN967389, ABIN968537
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	250 µg/mL
Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09 % 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:	-20 °C
Storage Comment:	Store undiluted at -20°C.

Publications

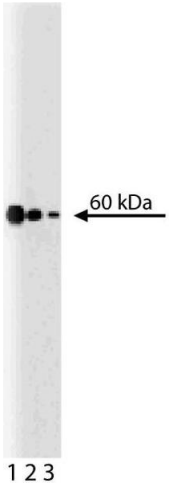
Product cited in:	Straub, Grue, Uhse, Lisby, Knudsen, Tange, Westergaard, Boege: "The RNA-splicing factor PSF/p54 controls DNA-topoisomerase I activity by a direct interaction." in: **The Journal of biological chemistry**, Vol. 273, Issue 41, pp. 26261-4, (1998) (PubMed). Basu, Dong, Krainer, Howe: "The intracisternal A-particle proximal enhancer-binding protein activates transcription and is identical to the RNA- and DNA-binding protein p54nrb/NonO." in:

Publications

Molecular and cellular biology, Vol. 17, Issue 2, pp. 677-86, (1997) (PubMed).

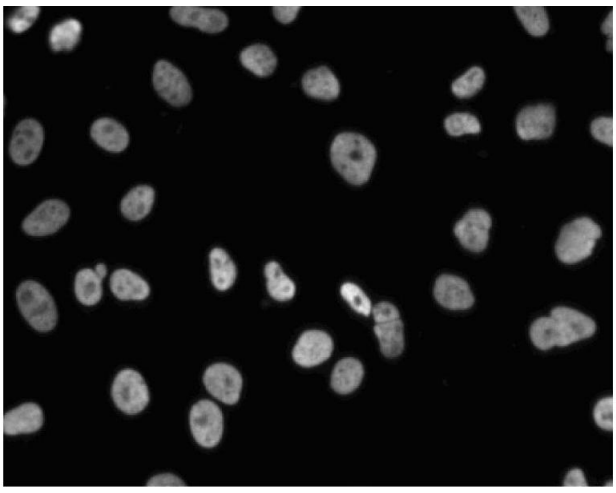
Dong, Horowitz, Kobayashi, Krainer: "Purification and cDNA cloning of HeLa cell p54nrb, a nuclear protein with two RNA recognition motifs and extensive homology to human splicing factor PSF and Drosophila NONA/BJ6." in: **Nucleic acids research**, Vol. 21, Issue 17, pp. 4085-92, (1993) (PubMed).

Images



Western Blotting

Image 1. Western blot analysis of p54 [nrb] on a Jurkat lysate. Lane 1: 1:2500, lane 2: 1:5000, lane 3: 1:10000 dilution of the p54 [nrb] antibody.



Immunofluorescence

Image 2. Immunofluorescent staining of A549 (ATCC CCL-185) cells. Cells were seeded in a 96 well imaging plate at ~ 10 000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the anti-p54 [nrb] antibody. The second step reagent was FITC goat anti mouse Ig. The image was taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained HeLa (ATCC CCL-2) and U-2 OS (ATCC HTB-96) cells using both the Triton™ X-100 and alcohol perm protocols.