Technical Data Sheet

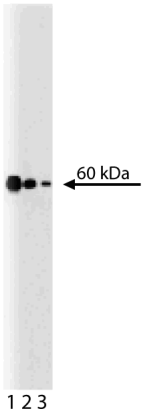
Purified Mouse Anti-p54 [nrb]

Product Information

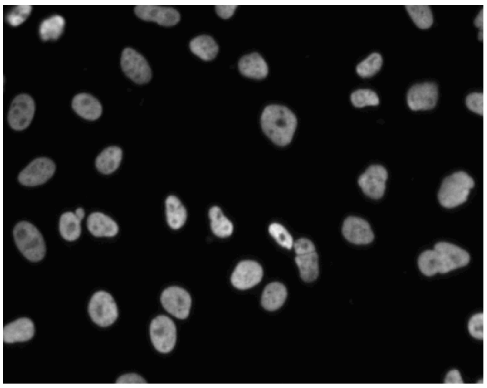
Material Number:	**611279**
Size:	150 µg
Concentration:	250 µg/ml
Clone:	3/p54nrb
Immunogen:	Human p54 [nrb] aa. 368-471
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Dog, Mouse, Rat
Target MW:	60 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

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] (**n**uclear **R**NA-**b**inding **p**rotein, **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.



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.*



Immunofluorescent staining of A549 (ATCC CCL-185) cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) 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 (Cat. No. 554001). 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 (see Recommended Assay Procedure).*

Preparation and Storage

Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl 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 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl 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 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp
Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611451	Jurkat Cell Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols 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 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.

References

Basu A, Dong B, Krainer AR, Howe CC. The intracisternal A-particle proximal enhancer-binding protein activates transcription and is identical to the RNA- and DNA-binding protein p54nrb/NonO. *Mol Cell Biol.* 1997; 17(2):677-686. (Biology)

Dong B, Horowitz DS, Kobayashi R, Krainer AR. 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. *Nucleic Acids Res.* 1993; 21(17):4085-4092. (Biology)

Straub T, Grue P, Uhse A, et al. The RNA-splicing factor PSF/p54 controls DNA-topoisomerase I activity by a direct interaction. *J Biol Chem.* 1998; 273(41):26261-26264. (Biology)