Product Datasheet

hnRNP M Antibody (2A6)
NB200-315

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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Publications: 3

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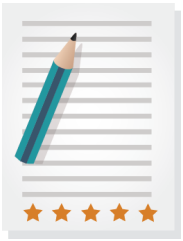


NB200-315

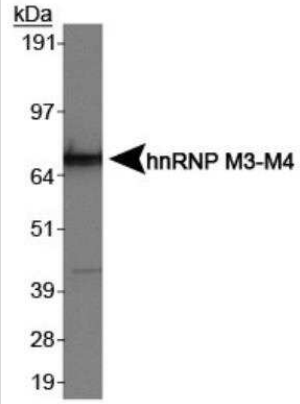
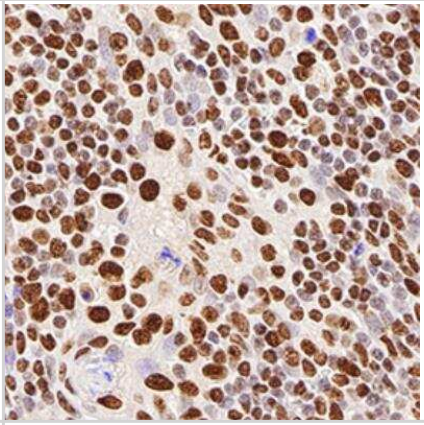
hnRNP M Antibody (2A6)

Product Information	
Unit Size	0.1 ml
Concentration	This product is unpurified. The exact concentration of antibody is not quantifiable.
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	2A6
Preservative	0.1% Sodium Azide
Isotype	IgG2b Kappa
Purity	Unpurified
Buffer	Ascites
Target Molecular Weight	68 kDa

Product Description	
Host	Mouse
Gene Symbol	HNRNPM
Species	Human, Mouse, Rat, Porcine, Bovine, Rabbit
Specificity/Sensitivity	NB200-315 is specific for hnRNP M3 and M4 proteins.
Immunogen	M19 fusion protein containing full-length human protein. [UniProt# P52272]

Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:3000, Immunohistochemistry 1:10-1:500, Immunocytochemistry/ Immunofluorescence 1:10-1:500, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:10-1:500, Immunohistochemistry-Frozen 1:10-1:500
Application Notes	This hnRNP M (2A6) antibody is useful for Immunocytochemistry/Immunofluorescence, Immunohistochemistry on paraffin-embedded and frozen sections, Immunoprecipitation and Western Blot. A 68 kDa band can be seen in a Western blot using HeLa cell lysate. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

Images

Western Blot: hnRNP M Antibody (2A6) [NB200-315] - Analysis of hnRNP M3-M4 in HeLa cell lysate.	
Immunohistochemistry-Paraffin: hnRNP M Antibody (2A6) [NB200-315] - IHC analysis of formalin fixed paraffin-embedded (FFPE) human tonsil using hnRNP M (2A6) antibody at 1:500 on a Bond Rx autostainer (Leica Biosystems). The assay involved 20 minutes of heat induced antigen retrieval (HIER) using 10mM sodium citrate buffer (pH 6.0) and endogenous peroxidase quenching with peroxide block. The sections were incubated with primary antibody for 30 minutes and Bond Polymer Refine Detection (Leica Biosystems) with DAB was used for signal development followed by counterstaining with hematoxylin. Whole slide scanning and capturing of representative images was performed using Aperio AT2 (Leica Biosystems). Nuclear riboprotein staining was observed. Staining was performed by Histowiz.	
Simple Western: hnRNP M Antibody (2A6) [NB200-315] - Simple Western lane view shows a specific band for hnRNP M in 0.05 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	

Publications

Sikorski K, Mehta A et al. A high-throughput pipeline for validation of antibodies. Nat Methods 2018-01-11 [PMID: 30377371] (Human)

Details:
Antibody validation based on denaturing gel electrophoresis of biotinylated cell lysates (PAGE) followed by mass spectrometry (MS) and antibody array analysis (MAP).

Vautier D, Chesne P, Cunha C, Calado A, Renard JP, Carmo-Fonseca M. Transcription-dependent nucleocytoplasmic distribution of hnRNP A1 protein in early mouse embryos. J Cell Sci;114(Pt 8):1521-31. 2001-04-01 [PMID: 11282028] (ICC/IF, Mouse)

Datar KV, Dreyfuss G, Swanson MS. The human hnRNP M proteins: identification of a methionine/arginine-rich repeat motif in ribonucleoproteins. Nucleic Acids Res;21(3):439-46. 1993-02-11 [PMID: 8441656] (WB, Mouse, Human)

Procedures

Western Blot protocol specific for hnRNP M3-M4 Antibody (NB200-315)
hnRNP M Antibody (2A6):
Western Blot Procedure

1) Wet Nitrocellulose membrane with PBS + NP40 [PN].
2) Pour off PN.
3) Dilute anti-RNP M3/M4 (catalog# NB 200-315) in 5%NFDM + NP40.
4) Incubate the primary antibody with the membrane for 1 hour, at room temperature (RT), gently rocking.
5) Wash 1x with PN for 10 minutes. Wash 2x with PN for 5 minutes, each.
6) Dilute anti-mouse-HRP (Amersham) in 5%NFDM + NP40 at 1:5,000.
7) Incubate the secondary antibody with the membrane for 45 minutes, at RT, gently rocking.
8) Wash 1x with PN for 10 minutes. Wash 2x with PN for 5 minutes, each.
9) Wash 1x with PBS for 5 minutes.
10) Develop using Amersham ECL components.

**NOTE: HeLa nuclear cell extracts can be used as a positive control for this antibody.

Immunocytochemistry/Immunofluorescence protocol for hnRNP M Antibody (NB200-315)
hnRNP M Antibody (2A6):
Day 1 Cell Growth

1. Grow cells directly on a 10-well microscope slide (Cel-Line Associates, 10-well, 7MM, HTC autoclavable slides, Catalog# 10-7) in 15 cm cell culture plates, overnight.
- The flatter the cells, the better the IF.
- HeLa JW36 cells are a better positive control than HeLa S3 cells.

Day 2 Fixation

2. Rinse slides 3x with PBS (swirl ~200 ml into a 250 ml beaker).
3. Fix slide in Coplin jar for 30 minutes at RT, in 2% formaldehyde (MeOH-free, 10% ultrapure, EM grade).
- Formaldehyde is diluted in PBS.
4. Rinse slides 3x with PBS (swirl 200 ml into a 250 ml beaker).
5. Incubate for 3 minutes in acetone at 20C (acetone stock should be kept at 20 degrees C).
6. Rinse slides 3x with PBS (swirl 200 ml into a 250 ml beaker).
7. Store fixed slides up to 1 week in PBS + 0.2% azide, in Coplin jar.

Same Day Immunofluorescence

8. Rinse slide 1x in PBS.
9. Dry with Kimwipe (or equivalent) and wipe around well with a cotton-tipped applicator.

**This drying procedure is repeated at each subsequent step, but be careful not to dry out the well

10. Add 10 ul of 3% BSA in PBS, per well, and pre-incubate for 15 minutes at RT in a humidifier chamber.
11. Wash slide 1x in PBS, add 10 ul of anti-RNP M1-M4 and incubate 60 minutes in chamber at RT.
12. Rinse slides 3x with PBS.
13. Add 10 ul of appropriate secondary antibody and incubate for 30 minutes at RT.
14. Rinse slides 3x in PBS.
15. Mount slides with IF mounting media and seal with clear nail polish.
16. Slides can be stored up to 1 week at 20C, in Revco box.

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Products Related to NB200-315

NBL1-11650	hnRNP M Overexpression Lysate
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-43317-0.5mg	Mouse IgG2b Kappa Light Chain Isotype Control (MG2b)

Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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