Product Datasheet

Mre11 Antibody
NB100-142

Unit Size: 0.05 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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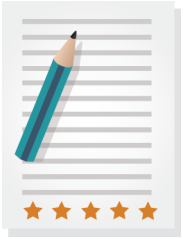
Updated 2/17/2025 v.20.1



NB100-142

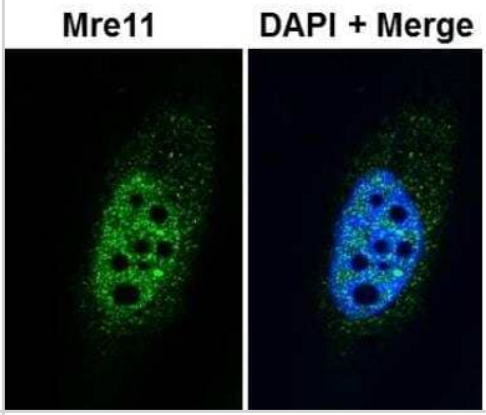
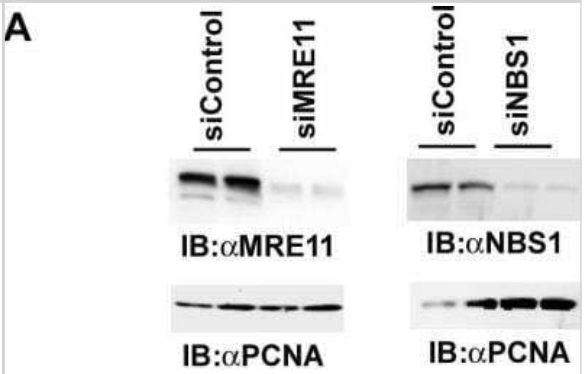
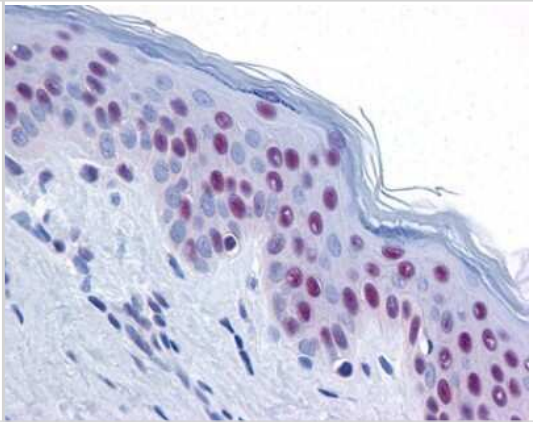
Mre11 Antibody

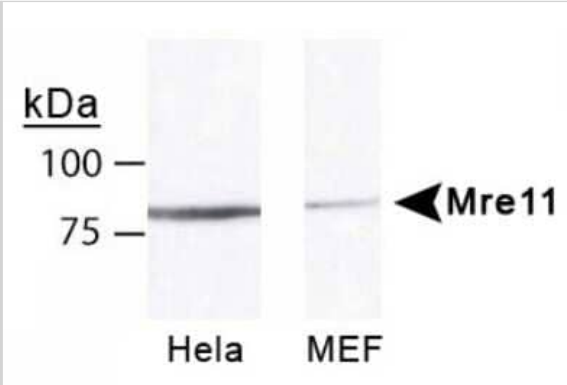
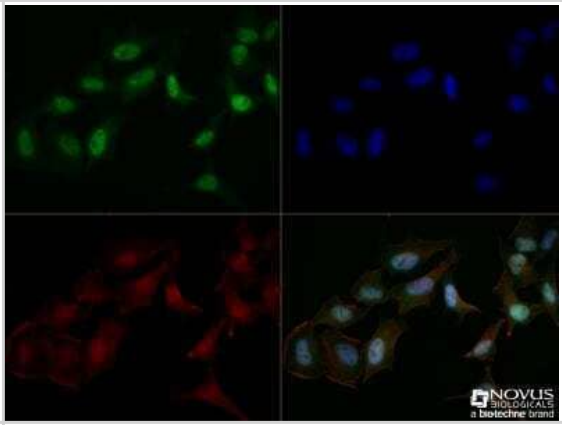
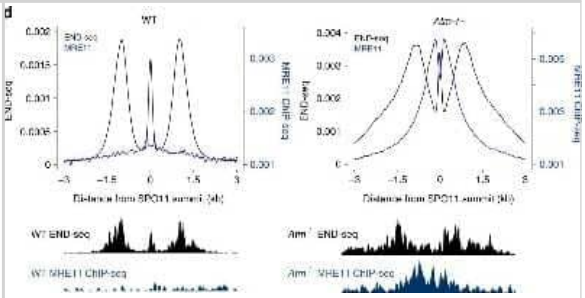
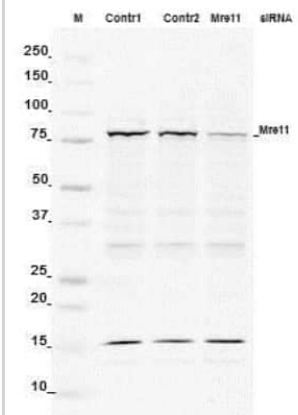
Product Information	
Unit Size	0.05 ml
Concentration	This product is unpurified. The exact concentration of antibody is not quantifiable.
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Unpurified
Buffer	Whole antisera
Target Molecular Weight	81 kDa

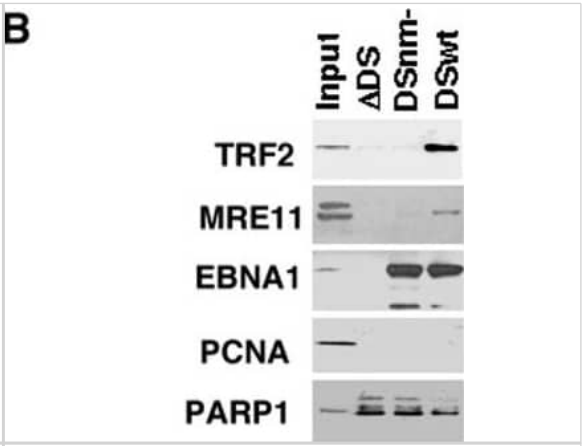
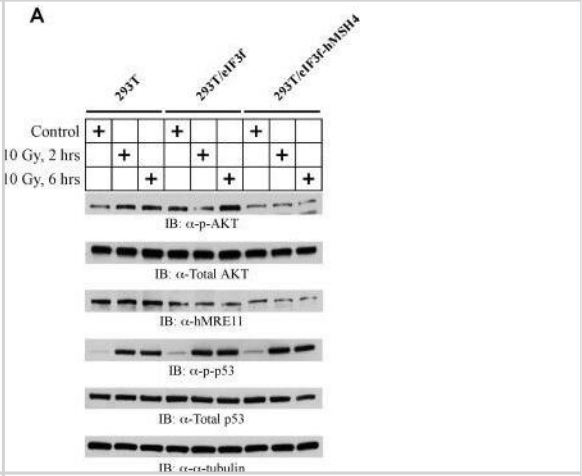
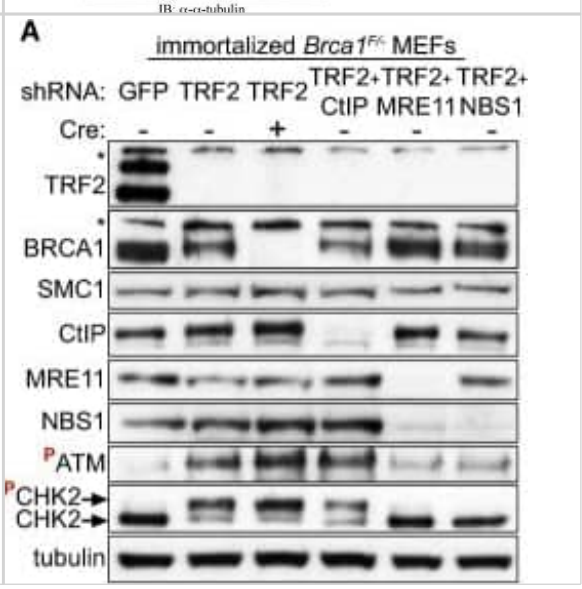
Product Description	
Host	Rabbit
Gene ID	4361
Gene Symbol	MRE11
Species	Human, Mouse, Rat, Chicken, Hamster
Reactivity Notes	Predicted cross-reactivity based on sequence identity: Gorilla (100%), Chimpanzee (100%), Gibbon (99%), Marmoset (96%), Canine (94%), Feline (94%), Panda (94%), Equine (92%), Bovine (92%), Bat (92%).
Immunogen	Mre11 Antibody is made to a full length human Mre11 protein. [Uniprot: P49959]

Product Application Details	
Applications	Western Blot, Simple Western, ELISA, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Proximity Ligation Assay, Chromatin Immunoprecipitation (ChIP), Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 1:5000, Simple Western 1:250, Flow Cytometry, ELISA reported in scientific literature (PMID 16788144), Immunohistochemistry 1:10 - 1:500, Immunocytochemistry/ Immunofluorescence 1:200. Use reported in scientific literature (PMID 26774475), Immunoprecipitation 3 uL, Immunohistochemistry-Paraffin 1:10 - 1:500. Use reported in scientific literature (PMID 21279473), Immunohistochemistry-Frozen reported in scientific literature (PMID 24349281), Immunoblotting reported in scientific literature (PMID 28115467), Proximity Ligation Assay reported in scientific literature (PMID 32780723), Chromatin Immunoprecipitation (ChIP), Knockout Validated reported in scientific literature (VanCevska et al), Knockdown Validated
Application Notes	In Western blot, a band can be seen at ~ 81 kDa. For ICC/IF, this antibody has been used with methanol-fixed IMR90 primary human fibroblasts. For IP, the suggested working dilution is 3 ul for immunoprecipitation of 3X10^6 cells. Co-IP application has been reported in scientific literature (PMID: 22190719) In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:250, apparent MW was 102 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.

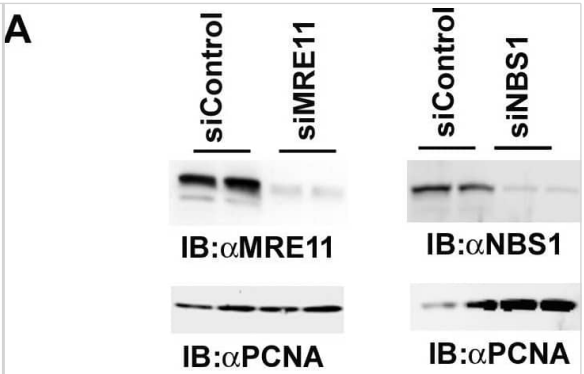
Images

Simple Western: Mre11 Antibody [NB100-142] - Image shows a specific band for Mre11 in 0.5 mg/mL of HeLa lysate. A band appears at the theoretical molecular weight of 81 kDa for this product. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Immunocytochemistry/Immunofluorescence: Mre11 Antibody [NB100-142] - IF analysis of Mre11 on HeLa. Image from verified customer review.	
Western Blot: Mre11 Antibody [NB100-142] - Mre11 and NBS1 contribute to OriP replication and EBV episome stability. D98/HR1 cells were transfected with siRNA for Mre11 or NBS1, or Luciferase control and assayed by immunoblot (IB) with antibodies specific for Mre11 (left pane) or NBS1 (right panel), or PCNA (lower panel) as a loading control. Image collected and cropped by CiteAb from the following publication (https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0001257) licensed under a CC-BY license.	
Immunohistochemistry: Mre11 Antibody [NB100-142] - Immunohistochemical staining of MRE11 in a human epidermis cross section.	

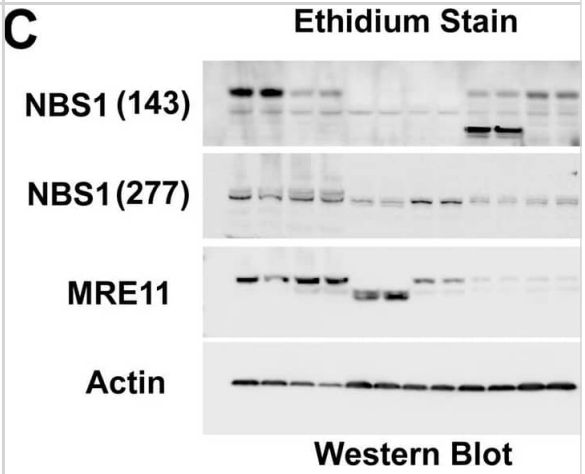
Western Blot: Mre11 Antibody [NB100-142] - Western blot analysis of Mre11 on 50 ug of HeLa and MEF lysates, displaying bands at the molecular weight of 81 kDa.	
Immunocytochemistry/Immunofluorescence: Mre11 Antibody [NB100-142] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-Mre11 [NB100-142] at a 1:500 dilution overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.	
Chromatin Immunoprecipitation: Mre11 Antibody [NB100-142] - Aggregated END-seq signal and MRE11 ChIP-seq RPM in WT (left) and Atm -/- (right). To fairly compare ChIP-seq signal between WT and Atm -/-, MRE11 scale is proportional to spike-in normalized END-seq RPM for each genotype. Individual hotspot examples (chr12:34,592,264-34,598,265) are shown below. Note that decreased MRE11 coverage is observed within NDR of Atm -/-. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/32051414/) licensed under a CC-BY license.	
Western Blot: Mre11 Antibody [NB100-142] - TIG-1 human primary fibroblasts, whole cell lysate (30 ug). Image from verified customer review.	

Western Blot: Mre11 Antibody [NB100-142] - MRE11 associates with the DS region of OriP.A) DNA affinity purification assays were performed with Raji nuclear extracts & DNA templates derived from DS wt, DS nm-, or ΔDS. Input & template bound proteins were analyzed by Western blot with antibodies to TRF2, MRE11, EBNA1, PCNA, & PARP1, as indicated. B) Schematic of template DNA used for DNA affinity purification in A, & their respective protein binding sites. C) ChIP assays to monitor EBNA1, TRF2, & MRE11 binding at OriP wt or OriP nm- in transfected 293 cells. Immunoprecipitated DNA was quantified by real time PCR relative to total input DNA recovered for each transfected cell. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/18040525), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: Mre11 Antibody [NB100-142] - Immunoblotting analysis of IR-induced AKT (Ser473) activation. (A) The levels of AKT activation were measured by AKT Ser473 phosphorylation in 293T, 293T/eIF3f & 293T/eIF3f-hMSH4 cells treated with 10 Gy IR in comparison to untreated controls. Levels of p53 Ser15 phosphorylation & the total protein levels of AKT, hMRE11 & p53 were also analyzed. α-tubulin was used as a loading control. (B) The levels of Chk2 activation (Chk2 Thr68 phosphorylation) in 293T, 293T/eIF3f & 293T/eIF3f-hMSH4 cells in response to 10 Gy IR. Cell lysates were prepared at 1 hr post IR treatment. Untreated cells were analyzed as controls, while α-tubulin was used as a loading control. Image collected & cropped by CiteAb from the following publication (https://molecular-cancer.biomedcentral.com/articles/10.1186/1476-4598-12-51), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: Mre11 Antibody [NB100-142] - The DNA damage response at telomeres uncapped through TRF2 depletion does not require BRCA1 or CtIPImmortalized Brca1F/− MEFs were infected with retroviruses expressing the indicated shRNAs and/or Cre recombinase, followed by selection with puromycin for 72 h. Cell extracts were prepared 48 h later & analysed by Western blotting as indicated. SMC1 & tubulin were used as loading controls. *non-specific band.Cells treated as in (A) were fixed 48 h after selection & stained with an anti-53BP1 antibody (green). Telomeres were visualized with a Cy3-conjugated (CCCTAA)3-PNA probe (red). Yellow arrowheads point to 53BP1 foci that co-localize with telomeres.Quantification of TIFs in cells treated as in (B). A minimum of 200 nuclei were scored for each sample. Error bars represent SD of two independent experiments. P-values were calculated using an unpaired two-tailed t-test. *P ≤ 0.05; NS, P > 0.05. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25582120), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

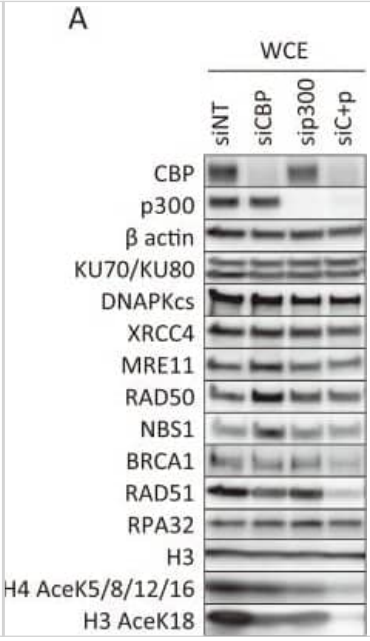
Western Blot: Mre11 Antibody [NB100-142] - MRE11 & NBS1 contribute to OriP replication & EBV episome stability.A) D98/HR1 cells were transfected with siRNA for MRE11 or NBS1, or Luciferase control & assayed by immuno-blot (IB) with antibodies specific for MRE11 (left pane) or NBS1 (right panel), or PCNA (lower panel) as a loading control. B) Transient DNA replication assays of OriP-containing plasmids were analyzed by Southern blot. D98/HR1 cells were transfected with OriP plasmid & siRNA for either MRE11, NBS1, or luciferase control (as indicated above each lane). Hirt extracted plasmid DNA was digested with DpnI plus BamHI (top panel) or BamHI only (lower panel) & probed with OriP specific probes. DNA was quantified by PhosphorImager analysis & the replication value was determined as the ratio of DpnI/BamHI to BamHI recovered products. Quantification shown below is a summary of at least four independent replication assays (data not shown). C)) EBV episomes from latently infected D98/HR1 cells were analyzed by pulse field electrophoresis & Southern blotting after transfection of siRNA for control (siLuc), MRE11, or NBS1. Raji cells were used as a control for EBV episome size & abundance. The percentage of EBV episomes relative to the EBV DNA retained in the well is calculated below. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/18040525), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Mre11 Antibody [NB100-142] - Evidence that MRE11 & NBS1 are required for EBV episome maintenance.A) OriP plasmid maintenance assays were performed in MRE11 mutant (ATLD3) & reconstituted (ATLD3/wtMRE11) cell lines (left panel) or NBS1 mutant (GM7166) & reconstituted (GM7166/wtNBS1) cell lines (right panel). Plasmids containing OriP & EBNA1 were monitored by Southern blotting of Hirt lysates at 1 (lower panel) & 7 (upper panel) days post-transfection. Phosphorimager quantification of four independent experiments as shown in panal A where maintenance is measured as the ratio of day 7 to day 1 for OriP plasmid detection is shown below. B) EBV transformed B-lymphocytes were analyzed by PFG & Southern blotting for the presence of episomal forms of the EBV genome. Raji, Namalwa, NBS1 (GM15808), NBS1 (GM07078), LCL3472, & LCLAW7 DNA was loaded at equal concentrations & analyzed by ethidium staining of PFG (lower panel) or by Southern blot (upper panel) with EBV specific probe. C) Western blot of the same cell lines used for PFG analysis shown in panel B was probed with antibodies specific for NBS1 (antibody 143 & antibody 277), MRE11, or loading control Actin. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/18040525), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Mre11 Antibody [NB100-142] - Involvement of CBP & p300 in the transcription of the BRCA1 & RAD51 genes.(A) Down-regulation of BRCA1 & RAD51 proteins upon depletion of CBP and/or p300. H1299 cells were transfected for 48 hr with non-targeting (siNT), CBP (siCBP), p300 (sip300), or CBP+p300 (siC+p) siRNAs. The cells were harvested & whole cell extracts were subjected to immunoblotting. (B, C) Reduction of BRCA1 & RAD51 transcripts in CBP- & p300-depleted cells. H1299 cells were transfected for 48 hr with non-targeting (siNT), CBP (siCBP), p300 (sip300), or CBP+p300 (siC+p) siRNAs. Cells were harvested & subjected to quantitative real-time PCR for the detection of BRCA1 (B) & RAD51 (C) mRNAs. Expression levels were normalized against the levels of GAPDH mRNA. Data represent the mean ± SD. (D) H1299 cells were transfected for 48 hr with non-targeting (siNT), CBP (siCBP), p300 (sip300), or CBP+p300 (siC+p) siRNAs, & stained with propidium iodide (PI). The percentage of cells in each cell cycle phase was determined by FACS. Percentages of cells in G1, S, & G2/M are shown. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23285190), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Elango R, Nilavar NM, Li AG, Nguyen D et Al. Two-ended recombination at a Flp-nickase-broken replication fork Mol Cell 2024-12-04 [PMID: 39631396]

Kim JJ, Lee SY, Choi JH et al. PCAF-Mediated Histone Acetylation Promotes Replication Fork Degradation by MRE11 and EXO1 in BRCA-Deficient Cells Mol. Cell 2020-09-20 [PMID: 32966758]

Whelan DR, Rothenberg E., et Al. Super-resolution mapping of cellular double-strand break resection complexes during homologous recombination Proc Natl Acad Sci U S A 2021-03-16 [PMID: 33707212]

Ubieto-Capella P, Ximénez-Embún P, Giménez-Llorente D et Al. A rewiring of DNA replication mediated by MRE11 exonuclease underlies primed-to-naive cell de-differentiation Cell Rep 2024-04-05 [PMID: 38581679]

Lebdy R, Canut M, Patouillard J et Al. The nucleolar protein GNL3 prevents resection of stalled replication forks EMBO Rep 2023-12-11 [PMID: 37965896]

DR Whelan, WTC Lee, F Marks, YT Kong, Y Yin, E Rothenberg Super-resolution visualization of distinct stalled and broken replication fork structures PloS Genetics, 2020-12-28;16(12):e1009256. 2020-12-28 [PMID: 33370257]

Baldwin JG, Heuser-Loy C, Saha T et Al. Intercellular nanotube-mediated mitochondrial transfer enhances T cell metabolic fitness and antitumor efficacy Cell 2024-09-12 [PMID: 39276774]

I Paniagua, Z Tayeh, M Falcone, S Hernández, A Cerutti, JJL Jacobs MAD2L2 promotes replication fork protection and recovery in a shieldin-independent and REV3L-dependent manner Nature Communications, 2022-09-08;13 (1):5167. 2022-09-08 [PMID: 36075897]

Polyzos, AA;Cheong, A;Yoo, JH;Blagec, L;Toprani, SM;Nagel, ZD;McMurray, CT; Base excision repair and double strand break repair cooperate to modulate the formation of unrepaired double strand breaks in mouse brain Nature communications 2024-09-04 [PMID: 39231940]

Tang Z, Liang Z, Zhang B et al. MRE11 is essential for the long□term viability of undifferentiated spermatogonia Cell Proliferation 2024-06-18 [PMID: 38894566]

Joseph M Dybas, Krystal K Lum, Katarzyna Kulej, Emigdio D Reyes, Richard Lauman, Matthew Charman, Caitlin E Purman, Robert T Steinbock, Nicholas Grams, Alexander M Price, Lydia Mendoza, Benjamin A Garcia, Matthew D Weitzman Adenovirus Remodeling of the Host Proteome and Host Factors Associated with Viral Genomes. mSystems 2021-08-31 [PMID: 34463575]

Néstor García-Rodríguez, Iria Domínguez-García, María del Carmen Domínguez-Pérez, Pablo Huertas EXO1 and DNA2-mediated ssDNA gap expansion is essential for ATR activation and to maintain viability in BRCA1-deficient cells Nucleic Acids Research 2024-06-24 [PMID: 38721777]

More publications at http://www.novusbio.com/NB100-142

Procedures

Serum protocol for Mre11 Antibody (NB100-142)

Immunoprecipitation Procedure
1. For IP reactions, start with extract (whole cell or nuclear) from around 3 million cells prepared in 0.5-1 ml lysis buffer (100 mM NaCl, 10 mM Tris HCl, 5 mM EDTA, 0.5% nonidet p40).
2. Cells are resuspended in lysis buffer, then incubated with rotation about 15 min at 4 degrees C.
3. The lysate is then centrifuged 5 min at 14000g to remove insoluble material.
4. To cleared lysate, add 1-3 ul of antiserum and incubate on ice for 30 min.
5. Collect immune complexes on Protein A Sepharose by adding 25 ul of a 50% slurry, and incubate with rotation for 1 hour at 4 degrees C.
6. The complexes are pelleted gently (5000g for 5-10 sec.) then washed with 1 ml lysis buffer.
7. Repeat the wash 2 more times.
8. Analyze the immunoprecipitates by SDS PAGE. This antibody works well for IP reactions from both human and mouse cells. The intact complex is stable and can be immunoprecipitated in many common lysis buffers (up to 0.5 M NaCl).
Western Blot Procedure
1. Run 50 ug of protein on a 4-20% Tris-glycine mini-gel at 125V for 90 minutes.
2. Equilibrate gel, nitrocellulose membrane, Whatman paper, and blotting pads in transfer buffer for 15 minutes.
3. Transfer protein to the membrane at 25V for 90 minutes.
4. Allow membrane to air-dry.
5. Block membrane with 1XPBS/3% BSA for 1 hour at room temperature (23-27 degrees C).
6. Wash membrane twice, for 5 minutes each, with 1XPBS/0.05% Tween-20 (PBST).
7. Incubate membrane with 1:5000 dilution of NB100-142 (anti-hMre11), diluted in 1XPBS/1% BSA, for 1 hour at room temperature.
8. Wash membrane once for 15 minutes, then four times for 5 minutes each, with PBST.
9. Incubate membrane with goat anti-rabbit IgG-HRP, diluted in 1XPBS/1% BSA, for 1 hour at room temperature.
10. Wash membrane once for 15 minutes, then four times for 5 minutes each, with PBST.
11. Detect cross-reacting proteins using Renaissance Chemiluminescence Reagent Plus kit from NEN Life Sciences.
NOTE: HeLa whole cell extracts (NB800-PC1) were used as a positive control for this antibody.
Immunofluorescence Procedure
A 5beta in situ extraction method [10mM Pipes, pH 6.8 / 0.2% Triton X-100 / 100mM MgCl2 / 100mM sucrose/ 10mM EGTA Beta on ice] followed by 4% paraformaldehyde fixation of tissues works well for immunofluorescence of anti-hMre11 (NB 100-142).
Please see reference: Franchitto, A., Pichierri, P., Blooms syndrome protein is required for correct relocalization of RAD50/Mre11/nbs1 complex after replication fork arrest. J. of Cell Biology, DOI: 10 (2002)
Immunohistochemistry - FFPE sections
I. Deparaffinization:
A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
II. Quench Endogenous Peroxidase:
A. Place slides in peroxidase quenching solution: 15-30 minutes.
To Prepare 200 ml of Quenching Solution:
Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.
Use within 4 hours of preparation
B. Place slides in distilled water: 2 changes for 2 minutes each.
III. Retrieve Epitopes:
A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees Celcius.
B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
D. Slowly add distilled water to further cool for 5 minutes.E. Rinse slides with distilled water. 2 changes for 2 minutes each.
IV. Immunostaining Procedure:
A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).

B. Flood slide with Wash Solution. Do not allow tissue sections to dry for the rest of the procedure.
C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of primary antibody solution to each slide, and incubate for 1 hour.
E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.
I. Wash slides with Wash Solution: 3 changes for 5 minutes each.
J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
M. Rinse slides in distilled water.
N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.
S. Lay slides on a flat surface to dry prior to viewing under microscope.
NOTES:
Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
Prior to deparaffinization, heat slides overnight in a 60 degrees Celcius oven.
All steps in which Xylene is used should be performed in a fume hood.
For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.
For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.
200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.
5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.
Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1 1/2 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).

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