

Product Datasheet

Mre11 Antibody - BSA Free 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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NB100-142

Mre11 Antibody - BSA Free

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	
Description	Novus Biologicals Knockout (KO) Validated Rabbit Mre11 Antibody - BSA Free (NB100-142) is a polyclonal antibody validated for use in IHC, WB, ELISA, Flow, ICC/IF, Simple Western, IP and ChIP. Anti-Mre11 Antibody: Cited in 240 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
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, Immunohistochemistry-Paraffin, ELISA, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, 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

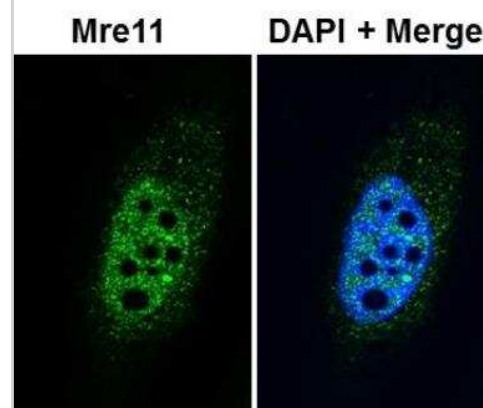
In Western blot, a band can be seen at ~ 81 kDa. For ICC/IF, this antibody has been used with methanol-fixed IMR90 primary human fibroblasts. For IP, the suggested working dilution is 3 ul for immunoprecipitation of 3×10^6 cells. Co-IP application has been reported in scientific literature (PMID: 22190719) 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

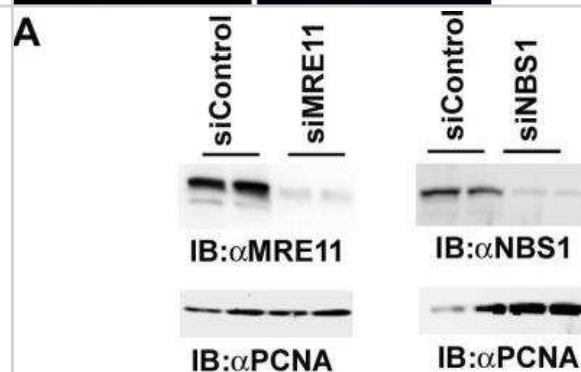
Simple Western: Mre11 Antibody [NB100-142] - Image shows a specific band for Mre11 in 0.5 mg/mL of HeLa lysate. A band appears at the theoretical molecular weight of 81 kDa for this product. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



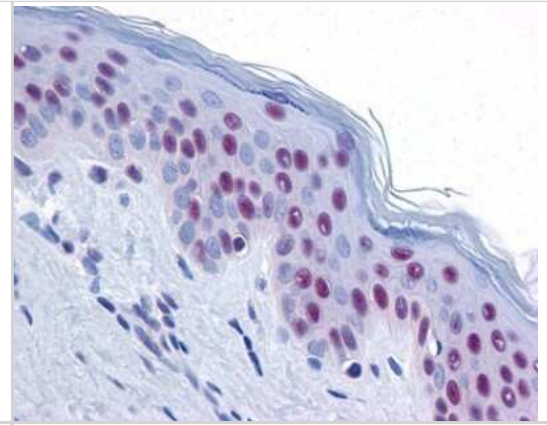
Immunocytochemistry/Immunofluorescence: Mre11 Antibody [NB100-142] - IF analysis of Mre11 on HeLa. Image from verified customer review.



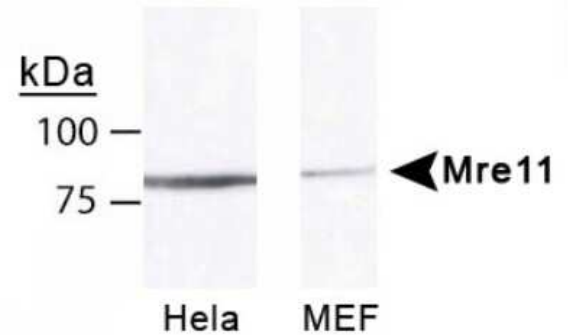
Western Blot: Mre11 Antibody [NB100-142] - Mre11 and NBS1 contribute to OriP replication and EBV episome stability. D98/HR1 cells were transfected with siRNA for Mre11 or NBS1, or Luciferase control and assayed by immunoblot (IB) with antibodies specific for Mre11 (left pane) or NBS1 (right panel), or 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.



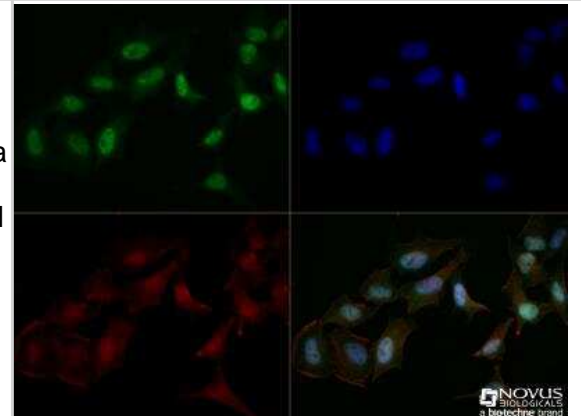
Immunohistochemistry: Mre11 Antibody [NB100-142] - Immunohistochemical staining of MRE11 in a human epidermis cross section.



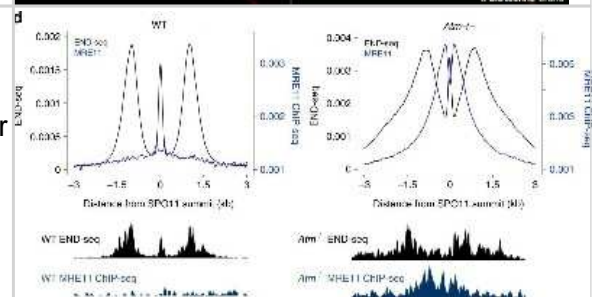
Western Blot: Mre11 Antibody [NB100-142] - Western blot analysis of Mre11 on 50 ug of HeLa and MEF lysates, displaying bands at the molecular weight of 81 kDa.



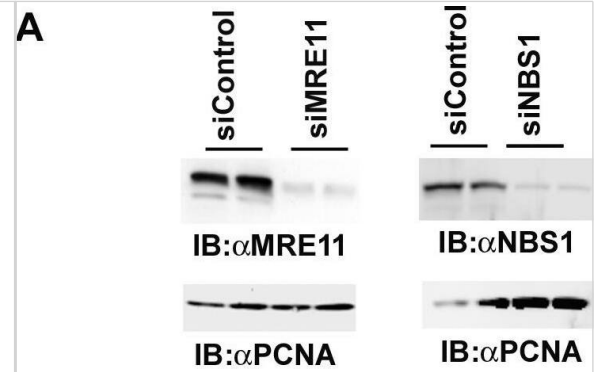
Immunocytochemistry/Immunofluorescence: Mre11 Antibody [NB100-142] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-Mre11 [NB100-142] at a 1:500 dilution overnight at 4C and detected with 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.



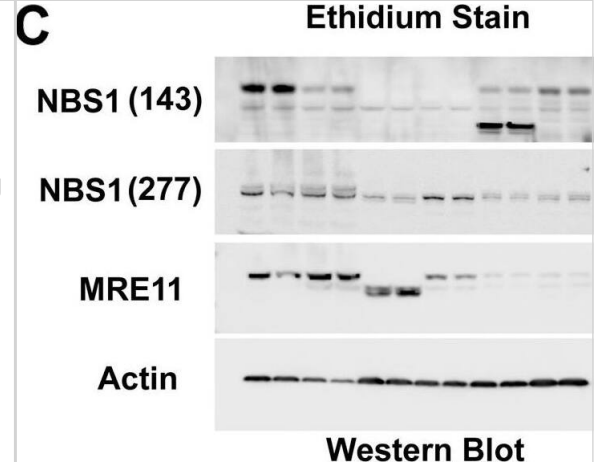
Chromatin Immunoprecipitation: Mre11 Antibody [NB100-142] - Aggregated END-seq signal and MRE11 ChIP-seq RPM in WT (left) and *Atm*^{-/-} (right). To fairly compare ChIP-seq signal between WT and *Atm*^{-/-}, MRE11 scale is proportional to spike-in normalized END-seq RPM for each genotype. Individual 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/](https://pubmed.ncbi.nlm.nih.gov/32051414/)) licensed under a CC-BY license.



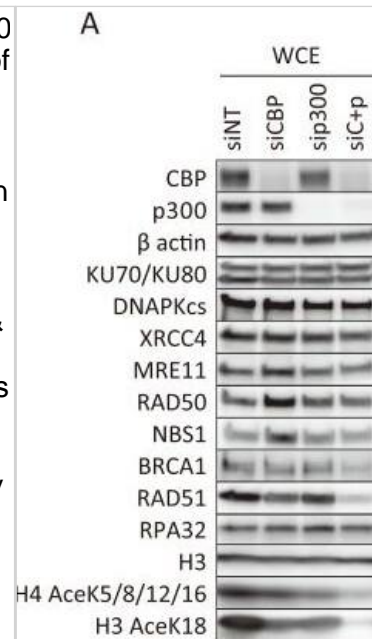
Western Blot: Mre11 Antibody [NB100-142] - MRE11 & NBS1 contribute to OriP replication & EBV episome stability. A) D98/HR1 cells were transfected with siRNA for MRE11 or NBS1, or Luciferase control & assayed by immuno-blot (IB) with antibodies specific for MRE11 (left pane) or NBS1 (right panel), or 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.



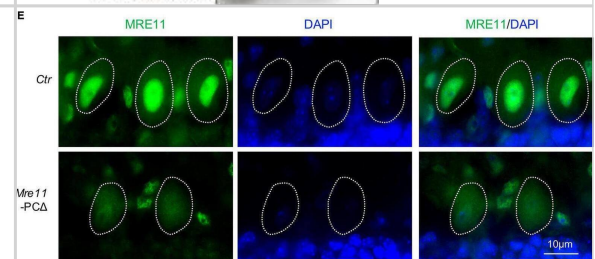
Western Blot: Mre11 Antibody [NB100-142] - Evidence that MRE11 & NBS1 are required for EBV episome maintenance. A) OriP plasmid maintenance assays were performed in MRE11 mutant (ATLD3) & reconstituted (ATLD3/wtMRE11) cell lines (left panel) or NBS1 mutant (GM7166) & reconstituted (GM7166/wtNBS1) cell 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 panel 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.



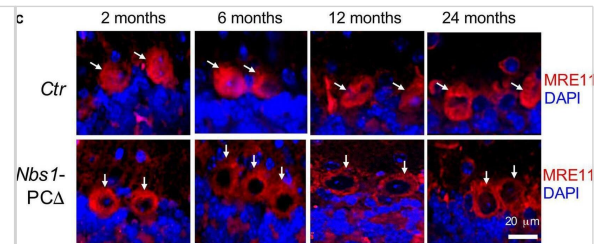
Western Blot: Mre11 Antibody [NB100-142] - Involvement of CBP & p300 in the transcription of the BRCA1 & RAD51 genes. (A) Down-regulation of BRCA1 & RAD51 proteins upon depletion of CBP and/or p300. H1299 cells were transfected for 48 hr with non-targeting (siNT), CBP (siCBP), p300 (sip300), or CBP+p300 (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 \pm 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.



Normal brain and the cerebellum of Mre11-PC Δ mice. (A) Abdominal view of wild type (Ctr) and Mre11-PC Δ mice showed normal limbs stretching. (B) Bodyweight of control (Ctr) and Mre11-PC Δ mice at 18 months of age. The number of mice analyzed is indicated within the graph bars. Error bars indicate the value mean \pm SEM; n.s., not significant; data comparison was performed through Student's t-test. (C) A dorsal view of the whole brain of control (Ctr) and Mre11-PC Δ mice. (D) Brain weight of 12-month-old Ctr and Mre11-PC Δ mice. The number of mice analyzed is indicated within the graph bars. Error bars indicate the value mean \pm SEM; data comparison was performed by Student's t-test. n.s., not significant. (E) Sagittal cryosections of 18-month-old Ctr and Mre11-PC Δ mice after staining with anti-MRE11 antibody and DAPI. Note a reduction of MRE11 signal in mutant Purkinje cells. The Purkinje cell layer in the cerebellum region is shown. Dotted lines highlight the nucleus of Purkinje cells. (F,G) Mre11-PC Δ mice have normal density and morphology of Purkinje cells. Sagittal sections of control (Ctr) and Mre11-PC Δ mice were stained with Calbindin-D28K (D28K) antibody (green) and DAPI (blue) to label Purkinje cells and nuclei, respectively (F). The upper panels show the whole cerebellum, and the lower panels show the magnified view of red frames in the respective upper panels. ML, molecular layer; PCL, Purkinje cell layer. (G) Quantification of total Purkinje cell number in each individual cerebellar lobule from panel (F). Three mice of each genotype were analyzed. Error bars indicate the value mean \pm SEM; data comparison was performed through Student's t-test. n.s., not significant. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/35153719>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Deletion of Nbs1 is compatible with mouse survival and cerebellar development. (A) Bodyweight of 20–24 months old mice from the indicated group. (B) Brain weight of the animal for panel (A), Error bars indicate the value mean \pm SEM; n.s., not significant. Data comparison was performed through Student's t-test. (C,D) Deletion of Nbs1 in Purkinje cells leads to dislocation of MRE11 and RAD50. Sagittal cryo-sections of control (Ctr) and Nbs1-PC Δ mice at the indicated age were stained with anti-MRE11 (C) and anti-RAD50 (D) antibodies together with DAPI to label nuclei. The Purkinje cell layer in the cerebellum region is shown. Scale bars: 20 μ m. Representative images of sections from two mice are shown. Arrows point to Purkinje cells. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/35153719>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

I Paniagua, Z Tayeh, M Falcone, S Hernández, A Cerutti, JLL 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] (Western Blot, Mouse)

Fowler FC, Chen BR, Zolnerowich N et al. DNA-PK promotes DNA end resection at DNA double strand breaks in G (0) cells eLife 2022-05-16 [PMID: 35575473] (Western Blot, Mouse)

Ding M, Qing X, Zhang G et al. The Essential DNA Damage Response Complex MRN Is Dispensable for the Survival and Function of Purkinje Neurons Frontiers in Aging Neuroscience 2022-01-28 [PMID: 35153719] (Western Blot, Mouse)

Zou RS, Liu Y, Gaido OER et al. Improving the sensitivity of in vivo CRISPR off-target detection with DISCOVER-Seq Nature Methods 2023-05-01 [PMID: 37024653] (Western Blot, Mouse)

Lazarchuk P, Nguyen VN, Brunon S et al. Innate immunity mediator STING modulates nascent DNA metabolism at stalled forks in human cells Frontiers in Molecular Biosciences 2023-01-12 [PMID: 36710880] (Western Blot, Mouse)

Price AM, Steinbock RT, Lauman R et al. Novel viral splicing events and open reading frames revealed by long-read direct RNA sequencing of adenovirus transcripts PLOS Pathogens 2022-09-12 [PMID: 36095031] (Western Blot, Mouse)

Einig E, Jin C, Andrioletti V et al. RNAPII-dependent ATM signaling at collisions with replication forks Nature Communications 2023-08-24 [PMID: 37620345] (Western Blot, Mouse)

SH Park, N Kang, E Song, M Wie, EA Lee, S Hwang, D Lee, JS Ra, IB Park, J Park, S Kang, JH Park, S Hohng, KY Lee, K Myung ATAD5 promotes replication restart by regulating RAD51 and PCNA in response to replication stress Nat Commun, 2019-12-16;10(1):5718. 2019-12-16 [PMID: 31844045] (Western Blot, Mouse)

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] (Western Blot, Mouse)

Lim KS, Li H, Roberts EA et al. USP1 Is Required for Replication Fork Protection in BRCA1-Deficient Tumors Mol. Cell 2018-12-20 [PMID: 30576655] (Western Blot, Mouse)

Deshpande RA, Marin-Gonzalez A, Barnes HK et al. Genome-wide analysis of DNA-PK-bound MRN cleavage products supports a sequential model of DSB repair pathway choice Nat Commun 2023-09-16 [PMID: 37717054] (Western Blot, Mouse)

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]

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

Procedures

Immunoprecipitation 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.

Immunocytochemistry/ Immunofluorescence Protocol for Mre11 Antibody (NB100-142)

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-Paraffin Protocol for Mre11 Antibody (NB100-142)

Standard protocol for IHC validation

Tissue Preparation: Formalin fixation and embedding in paraffin wax

The tissue needs to be fixed overnight in formalin at room temperature (approx. 16 hours).

There needs to be at least a 10:1 ratio of formalin volume to volume of the tissue.

The tissue can be no more than 4-5 mm thick. If it is thicker than this, it will need to be bivalved (bisected so as to make two similar separate parts) before being added to the formalin due to the diffusion coefficient of formalin.

The working area of the tissue cassettes is roughly 2.5 cm by 3.0 cm. So the cut tissue sample can be no larger than this.

Formalin refers to 10% neutral buffered formalin.

After formalin fixation overnight tissues can be processed according to the following protocol:

Tissue Sectioning: Make 4-um sections and place on pre-cleaned and charged microscope slides.

Heat in a tissue-dryingoven for 45 minutes at 60 degrees C.

Deparaffinization: Wash dry slides in 3 changes of xylene for 5 minutes each @ RT

Rehydration: Wash slides in 3 changes of 100% alcohol for 3 minutes each @ RT

Wash slides in 2 changes of 95% alcohol for 3 minutes each @ RT

Wash slides in 1 change of 80% alcohol for 3 minutes @ RT

Rinse slides in gentle running distilled water for 5 minutes @ RT

Antigen retrieval: Steam slides in 0.01 M sodium citrate buffer, pH 6.0 at 99-100 degrees C - 20 minutes

Remove from heat and let stand at room temperature in buffer - 20 minutes

Rinse in 1X TBS with Tween (TBST) at 1 minute @ RT

Immunostaining: (Do not allow tissues to dry at any time during the staining procedure)

Apply a universal protein block for 20 minutes @ RT

Drain protein block from slides, apply diluted primary antibody for 45 minutes @ RT

Rinse slides in 1X TBST - 1 minute @ RT

Apply a biotinylated anti-rabbit IgG (H+L) secondary for 30 minutes @ RT

Rinse slides in 1X TBST - 1 minute @ RT

Apply alkaline phosphatase streptavidin for 30 minutes @ RT

Rinse slides in 1X TBST - 1 minute @ RT

Apply alkaline phosphatase chromogen substrate for 30 minutes @ RT

Wash slides in distilled water for 1 minute @ RT

Dehydrate: (This method should only be used if the chromogen substrate is alcohol insoluble (e.g. Vector Red, DAB))

Wash slides in 2 changes of 80% alcohol for 1 minute each @ RT

Wash slides in 2 changes of 95% alcohol for 1 minute each @ RT

Wash slides in 3 changes of 100% alcohol for 1 minute each @ RT

Wash slides in 3 changes of xylene for 1 minute each @ RT

Apply coverslip



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Products Related to NB100-142

NB800-PC1	HeLa Whole Cell Lysate
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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