Store at -20°C

MRN Complex Antibody Sampler Kit

#8344

1 Kit (5 x 20 µl)

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

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Entrez-Gene ID #4361,
UniProt ID #P49959,

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Mre11 (31H4) Rabbit mAb	4847	20 µl	81 kDa	Rabbit IgG
Phospho-Mre11 (Ser676) Antibody	4859	20 µl	81 kDa	Rabbit IgG
Rad50 Antibody	3427	20 µl	153 kDa	Rabbit IgG
p95/NBS1 (D6J5I) Rabbit mAb	14956	20 µl	95 kDa	Rabbit IgG
Phospho-p95/NBS1 (Ser343) Antibody	3001	20 µl	95 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions, and additional application protocols.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:

Western blotting	1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Description: MRN Complex Antibody Sampler Kit offers an economical way of detecting each target protein. The kit contains enough primary and secondary antibody to perform two western blot experiments with each primary antibody.

Background: The Mre11-Rad50-Nbs1 (MRN) complex is a key mediator of genome maintenance, playing important roles in meiosis, telomere stability at the ends of chromosomes, and the cellular responses to DNA damage (1-5). Homodimers of the Mre11 and Rad50 subunits form a tetramer core that binds directly to DNA and associates with the Nbs1 subunit (6). The complex functions as a sensor of DNA damage and localizes to DNA double-strand breaks. At these DNA lesions, the MRN complex tethers DNA ends and processes free strands via the endonuclease and exonuclease activities of Mre11. In addition to stimulating both homologous recombination and nonhomologous end joining repair DNA pathways, MRN activates DNA damage checkpoint signaling cascades regulating cell cycle progression. In some contexts, MRN is required for ATM activation and downstream phosphorylation of p53, BRCA1, and Chk2 (7). ATM also phosphorylates Mre11, Rad50, and Nbs1 (also known as p95 and Nibrin). Notably, Nbs1 Ser343 and Mre11 Ser676 are phosphorylated by ATM. Phosphorylation modulates function and association with many mediators, some of which include 53BP1, RPA, hSSB1, TRF2, BRCA1, FANCD2, CtP1, Histone H2AX, MDC1, and WRN helicase. Each subunit is essential for mammalian embryonic development, as mice with homozygous-null mutations in Mre11, Nbs1, or Rad50 are lethal. Furthermore, MRN complex function is required in developing lymphocytes for antigen receptor gene recombination initiated by the Rag-1 and Rag-2 recombinases. In humans, Mre11 and Nbs1 mutations cause chromosomal instability and radiosensitivity and are associated with ataxia-telangiectasia-like disorder (ATLD) and Nijmegen breakage syndrome (NBS), respectively (8). Genomic instability and cancer have been shown to develop in cells with genetic mutations within MRN complex genes.

Specificity/Sensitivity: Antibodies detect endogenous levels of their respective proteins.

Source/Purification: Total polyclonal antibodies are produced by immunizing rabbits with synthetic peptides corresponding to the amino terminus of human Rad50 or surrounding Ala740 of human p95/NBS1 protein. Activation state-specific polyclonal antibodies are produced by immunizing rabbits with synthetic phosphopeptides corresponding to residues surrounding Ser343 of human p95/NBS1 or Ser676 of human Mre11. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Lys496 of human Mre11A.

Background References:

(1) D'Amours, D. and Jackson, S.P. (2002) *Nat Rev Mol Cell Biol* 3, 317-27.

(2) van den Bosch, M. et al. (2003) *EMBO Rep* 4, 844-9.

(3) Ajimura, M. et al. (1993) *Genetics* 133, 51-66.

(4) Deng, Y. et al. (2009) *Nature* 460, 914-8.

(5) Lamarche, B.J. et al. (2010) *FEBS Lett* 584, 3682-95.

(6) Williams, R.S. et al. (2009) *Cell* 139, 87-99.

(7) Uziel, T. et al. (2003) *EMBO J* 22, 5612-21.

(8) Zhao, S. et al. (2000) *Nature* 405, 473-7.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.