Store at –20°C

#9653

Double Strand Breaks (DSB) Repair Antibody Sampler Kit

☑ 1 Kit
(9 x 20 µl)

Cell Signaling TECHNOLOGY®

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rev. 06/16

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-ATM (Ser1981) (D6H9) Rabbit mAb	5883	20 µl	350 kDa	Rabbit IgG
Phospho-BRCA1 (Ser1524) Antibody	9009	20 µl	220 kDa	Rabbit IgG
DNA-PK Antibody	4602	20 µl	450 kDa	Rabbit IgG
Ku80 (C48E7) Rabbit mAb	2180	20 µl	86 kDa	Rabbit IgG
Mre11 (31H4) Rabbit mAb	4847	20 µl	81 kDa	Rabbit IgG
Phospho-p95/NBS1 (Ser343) Antibody	3001	20 µl	95 kDa	Rabbit IgG
Rad50 Antibody	3427	20 µl	153 kDa	Rabbit IgG
ATM (D2E2) Rabbit mAb	2873	20 µl	350 kDa	Rabbit IgG
XLF Antibody	2854	20 µl	39 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: The Double Strand Breaks (DSB) Repair Antibody Sampler Kit provides an economical means to investigate repair of double-strand DNA breaks within the cell. The kit includes enough antibody to perform two western blot experiments with each antibody.

Background: Double strand DNA breaks (DSB) in mammalian cells can be repaired by the related mechanisms of non-homologous end-joining (NHEJ) and homologous recombination (HR). A DNA-dependent protein kinase composed of DNA-binding subunits Ku70 and Ku86 and the DNA-PKcs catalytic subunit mediates NHEJ repair. The Ku heterodimer binds free DNA ends and recruits DNA-PKcs to the break (1). DNA-PKcs signals areas of DNA damage and recruits additional proteins, such as the Artemis exo- and endonuclease that processes and primes the damaged sequence (2,3). Following replacement DNA synthesis, a ligase complex composed of DNA ligase IV and XRCC4 joins the repaired ends. XRCC4-like factor (XLF) is an essential ligase-associated repair factor that promotes gap-filling during NHEJ (4).

Homologous recombination utilizes aligned homologous sequences as a repair template. The MRN complex, composed of Mre11, Rad50, and nibrin (p95/NBS1), plays a critical role in sensing, processing and repairing breaks (5). MRN interacts with BRCA1 and CtIP to facilitate 5' resection of DSB DNA to generate 3' ssDNA ends necessary for repair (6). DNA-binding protein Mre11 exhibits exonuclease and endonuclease activity and is largely responsible for ssDNA end processing (7). Interaction between the MRN complex and ATM kinase promotes association between the kinase and its substrates and likely leads to ATM activation (8). ATM acts a central controller of the cell cycle checkpoint by phosphorylating multiple targets, including c-Abl, BRCA1 and p95/NSB1. Activated c-Abl phosphorylates Rad52, which promotes Rad51 binding to ssDNA and subsequent annealing of ssDNA (7).

Specificity/Sensitivity: Each antibody in the Double Strand Breaks (DSB) Repair Antibody Sampler Kit detects endogenous levels of its respective protein and does not cross-react with other family members. Activation state antibodies only detect their target proteins when modified at the indicated site.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Lys496 of human Mre11A and the carboxy terminus of human Ku80. Activation state monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Ser1981 of human ATM. Polyclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to the carboxy-terminus of human DNA-PKcs, the amino terminus of human Rad50, the central sequence of human Rad52, and the carboxy terminus of human XLF. Activation state polyclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser1524 of human BRCA1 and surrounding Ser343 of human p95/NBS1. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Gottlieb, T.M. and Jackson, S.P. (1993) *Cell* 72, 131-42.
(2) Franco, S. et al. (2008) *J Exp Med* 205, 557-64.
(3) Collis, S.J. et al. (2005) *Oncogene* 24, 949-61.
(4) Akopiants, K. et al. (2009) *Nucleic Acids Res* 37, 4055-62.
(5) Williams, R.S. et al. (2007) *Biochem Cell Biol* 85, 509-20.
(6) Chen, L. et al. (2008) *J Biol Chem* 283, 7713-20.
(7) Czornak, K. et al. (2008) *J Appl Genet* 49, 383-96.
(8) Lee, J.H. and Paull, T.T. (2007) *Oncogene* 26, 7741-8.

U.S. Patent No. 5,675,063

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **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.

#9653

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.