

# DNA-PK Antibody

✓ 100 µl  
(10 Western mini-blot)



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TECHNOLOGY®

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new 11/03

Applications W	Species Cross-Reactivity H	Molecular Wt. 450 kDa	Source Rabbit
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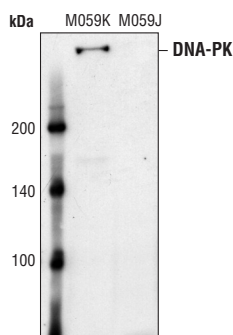
**Background:** DNA-dependent protein kinase (DNA-PK) is an important factor in the repair of double stranded breaks in DNA. Cells lacking DNA-PK or in which DNA-PK is inhibited fail to show proper non-homologous end-joining (NHEJ) (1–7). DNA-PK is composed of two DNA-binding subunits (Ku70 and Ku86) and one 450kDa catalytic subunit (DNA-PKcs) (8). It is thought that a heterodimer of Ku70 and Ku86 binds to double-stranded DNA broken ends before DNA-PKcs binds and is activated (1, 9). Activated DNA-PKcs is a serine/threonine kinase that has been shown to phosphorylate a number of proteins *in vitro*, including p53, transcription factors, RNA polymerase, and Ku70/Ku86 (10, 11). DNA-PKcs autophosphorylation at multiple sites, including threonine 2609, results in an inactivation of DNA-PK kinase activity and NHEJ ability (12, 13). It has been demonstrated, however, that DNA-PK preferentially phosphorylates substrates before it autophosphorylates, suggesting that DNA-PK autophosphorylation may play a role in disassembly of the DNA repair machinery (14, 15). Autophosphorylation at threonine 2609 has also been shown to be required for DNA-PK mediated double strand break repair, and phosphorylated DNA-PK co-localizes with H2A.X and 53BP1 at sites of DNA damage (16).

**Specificity/Sensitivity:** DNA-PK Antibody detects endogenous levels of DNA-PK protein.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to amino acids near the carboxy-terminus of human DNA-PKcs. Antibodies are purified using protein A and peptide affinity chromatography.

**Background References:**

- (1) Gottlieb, T.M. and Jackson, S.P. (1993) *Cell* 72, 131–142.
- (2) Hartley, K.O. et al. (1995) *Cell* 82, 840–856.
- (3) Rosenzweig, K.E. et al. (1997) *Clin. Cancer Res.* 3, 1149–1156.
- (4) Jackson, S.P. and Jeggo, P.A. (1995) *Trends Biochem. Sci.* 20, 412–415.



Western blot analysis of extracts from M059K (DNA-PK wildtype) and M059J (DNA-PK deficient) cells, using DNA-PK Antibody.

- (5) Roth, D.B. et al. (1995) *Curr. Biol.* 5, 496–499.
- (6) Baumann, P. and West, S.C. (1998) *Proc. Natl. Acad. Sci. USA* 95, 14066–14070.
- (7) Chen, S. et al. (2001) *J. Biol. Chem.* 276, 24323–24330.
- (8) Jeggo, P.A. (1997) *Mutat. Res.* 384, 1–14.
- (9) Suwa, A. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91, 6904–6908.
- (10) Anderson, C.W. and Lees-Miller, S.P. (1992) *Crit. Rev. Eukaryot. Gene Expr.* 2, 283–314.
- (11) Kuhn, A. et al. (1995) *Genes Dev.* 9, 193–203.
- (12) Chan, D.W. and Lees-Miller, S.P. (1996) *J. Biol. Chem.* 271, 8936–8941.
- (13) Douglas, P. et al. (2002) *Biochem. J.* 368, 243–251.
- (14) Lees-Miller, S.P. et al. (1992) *Mol. Cell. Biol.* 12, 5041–5049.
- (15) Jackson, S.P. et al. (1990) *Cell* 63, 155–165.
- (16) Chan, D.W. et al. (2002) *Genes Dev.* 16, 2333–2338.

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

**Recommended Antibody Dilutions:**

Western blotting 1:1000

**Companion Products:**

- Phospho-ATM (Ser1981) (10H11.E12) Monoclonal Antibody #4526
- Phospho-(Ser/Thr) ATM/ATR Substrate Antibody #2851
- Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- LumiGLO® Reagent and Peroxide #7003

**IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry F—Flow cytometry E—ELISA D—DELFIAP®  
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish All—all species expected  
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

## Western Immunoblotting Protocol

**For Western blots, incubate membrane with diluted anti-body in 5% BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

### A. Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

- A1. ■ 1X Phosphate Buffered Saline (PBS)
- A2. ■ 1X SDS Sample Buffer:  
62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- A3. ■ Transfer Buffer:  
25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- A4. ■ 10X Tris Buffered Saline (TBS):  
To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- A5. ■ Nonfat Dry Milk (weight to volume [w/v])
- A6. ■ Blocking Buffer:  
1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- A7. ■ Wash Buffer:  
1X TBS, 0.1% Tween-20 (TBS/T)
- A8. ■ Bovine Serum Albumin (BSA)
- A9. ■ Primary Antibody Dilution Buffer:  
1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- A10. ■ Phototope®-HRP Western Blot Detection System #7071:  
Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- A11. ■ Prestained Protein Marker, Broad Range (Premixed Format) #7720
- A12. ■ Biotinylated Protein Ladder Detection Pack #7727
- A13. ■ Blotting Membrane:  
This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B. Protein Blotting

A general protocol for sample preparation is described below.

- B1. Treat cells by adding fresh media containing regulator for desired time.
- B2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- B3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- B4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- B5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- B6. Microcentrifuge for 5 minutes.
- B7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

*Note: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.*

- B8. Electrotransfer to nitrocellulose or PVDF membrane.

### C. Membrane Blocking and Antibody Incubations

*Note: Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.*

- C1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- C2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- C3. Wash three times for 5 minutes each with 15 ml of TBS/T.
- C4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- C5. Wash three times for 5 minutes each with 15 ml of TBS/T.
- C6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- C7. Wash three times for 5 minutes each with 15 ml of TBS/T.

### D. Detection of Proteins

- D1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

*Note: LumiGLO® substrate can be further diluted if signal response is too fast.*

- D2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

*Note: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.*