#12311 Store at -20°C

DNA-PK (3H6) Mouse mAb

☑ 100 µl
(10 western blots)

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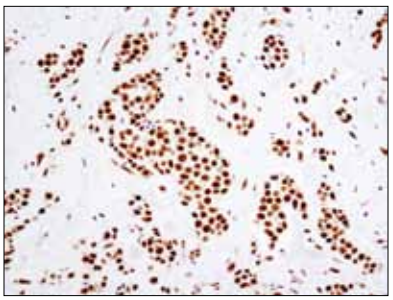
Entrez-Gene ID #5591
UniProt ID #P78527

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IHC-P, IF-IC	H, Mk	450 kDa	Mouse IgG1**
Endogenous			

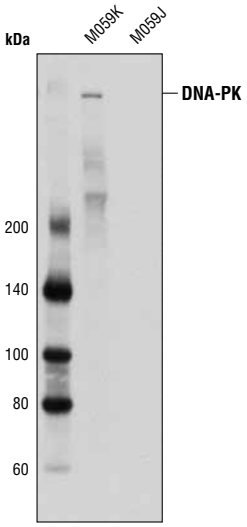
Background: DNA-dependent protein kinase (DNA-PK) is an important factor in the repair of double-stranded breaks in DNA. Cells lacking DNA-PK or in which DNA-PK is inhibited fail to show proper nonhomologous end-joining (NHEJ) (1-7). DNA-PK is composed of two DNA-binding subunits (Ku70 and Ku86) and one 450 kDa 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 Thr2609 and Ser2056, 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 Thr2609 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). Phosphorylation at Ser2056 occurs in response to double-stranded DNA breaks and ATM activation (17).

Specificity/Sensitivity: DNA-PK (3H6) Mouse mAb recognizes endogenous levels of total DNA-PK protein.

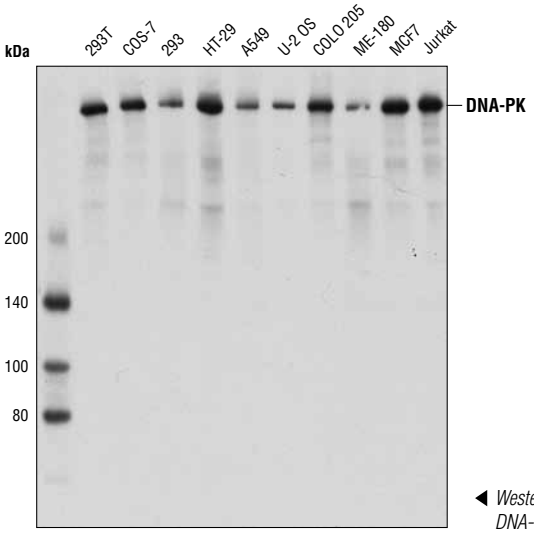
Source/Purification: Monoclonal antibody is produced by immunizing animals with a recombinant protein fragment specific to human DNA-PK protein expressed in *E.coli*.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma using DNA-PK (3H6) Mouse mAb.



Western blot analysis of extracts from M059K (DNA-PK wild-type) and M059J (DNA-PK deficient) cells using DNA-PK (3H6) Mouse mAb.



◀ *Western blot analysis of extracts from various cell lines using DNA-PK (3H6) Mouse mAb.*

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 antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-mouse secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunohistochemistry (Paraffin)	1:50†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Mouse) #8125

†*Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.*

Immunofluorescence (IF-IC)	1:100

For product specific protocols please see the web page for this product at www.cellsignal.com.

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

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

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.

Background References:

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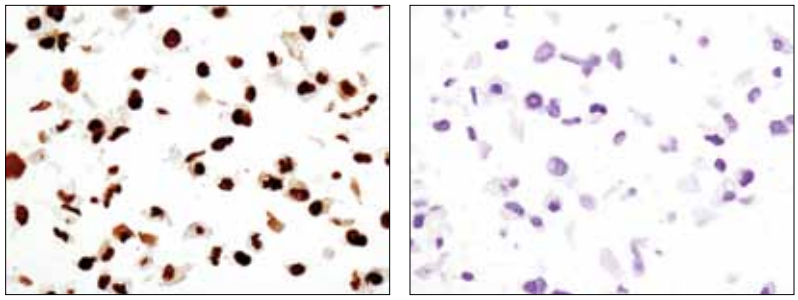
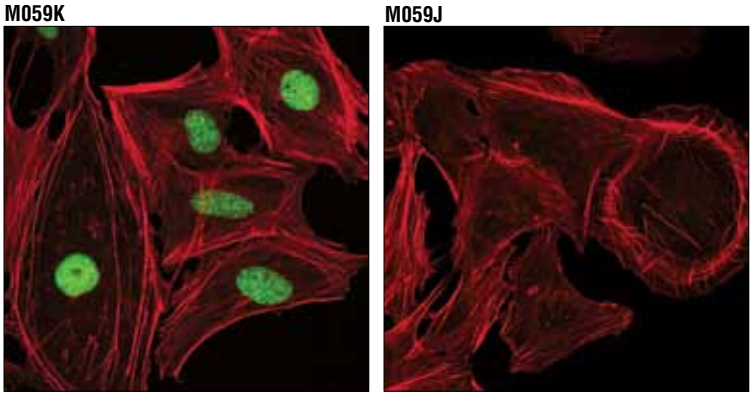


Immunohistochemical analysis of paraffin-embedded cell pellets, M059K (DNA-PK wild-type; left) or M059J (DNA-PK deficient, right), using DNA-PK (3H6) Mouse mAb.



Confocal immunofluorescent analysis of M059K (DNA-PK wild-type; left) and M059J (DNA-PK deficient; right) cells using DNA-PK (3H6) Mouse mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red).