Revision 5

Store at -20C

#38168

DNA-PKcs (E6U3A) Rabbit mAb

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3 Trask Lane | Danvers | Massachusetts | 01923 | USA

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IHC-P, IF-IC, FC-FP	H	Endogenous	450	Rabbit IgG	#P78527	5591

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunohistochemistry (Paraffin)	1:400 - 1:1600
Immunofluorescence (Immunocytochemistry)	1:50 - 1:100
Flow Cytometry (Fixed/Permeabilized)	1:100 - 1:400

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.

For a carrier free (BSA and azide free) version of this product see product #58268.

Specificity/Sensitivity

DNA-PKcs (E6U3A) Rabbit mAb recognizes endogenous levels of total DNA-PKcs protein.

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Pro608 of human DNA-PKcs protein.

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).

Background References

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12. Chan, D.W. and Lees-Miller, S.P. (1996) *J Biol Chem* 271, 8936-41.
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16. Chan, D.W. et al. (2002) *Genes Dev* 16, 2333-8.
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Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary 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 Blotting **IHC-P:** Immunohistochemistry (Paraffin) **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

Cross-Reactivity Key

H: Human

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