Technical Data Sheet

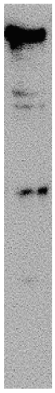
Purified Mouse Anti-Human DNA-PK

Product Information

Material Number:	**556456**
Alternate Name:	DNA Dependent Protein Kinase
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	4F10C5
Immunogen:	Human DNA-PK (p350) aa. 277-531 Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Target MW:	450-465 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The ability of mammalian cells to detect and repair DNA damage is essential in maintaining the structural integrity of their genes. Cells have evolved a number of repair pathways in order to repair different types of DNA lesions. DNA-dependent protein kinase (DNA-PK) is a serine-threonine protein kinase that is involved in DNA double-stranded break repair and variable-diversity-joining [V(D)J] recombination. To be active, DNA-PK must bind to double-stranded DNA containing broken ends, nicks, or single stranded gaps. Activated DNA-PK consists of three polypeptide components: Ku-80, Ku-70, and an ~350 kDa catalytic subunit (p350). First, Ku-80 and Ku-70 associate with each other to form a heterodimeric complex that binds to DNA breaks. By its interaction with DNA, Ku is thought to target p350 to sites of DNA damage. p350 then binds to the Ku-70/80 heterodimer (Ku) resulting in production of the active DNA-PK. Active DNA-PK phosphorylates a number of substrates in vitro, including proteins involved in the response of cells to DNA damage (p53, Ku, and replication protein A), and transcription factors (Sp1, Oct 1, Oct 2, SRF, Fos, and Jun). Clone 4F10C5 has been reported to recognize the 350 kDa catalytic subunit of DNA-PK.



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Western blot analysis for DNA-PK. *HeLa cell lysates (Human cervical epitheloid carcinoma; ATCC CCL-2) were probed with the mouse anti- DNA-PK antibody at 0.5 µg/mL (lane 1), 1.0 µg/mL (lane 2) and 2.0 µg/mL (lane 3). Although the catalytic subunit has been reported to be 350 kDa, DNA-PK may be observable migrating in a range of 450-465 kDa. Processed forms of the catalytic subunit may also be observed running lower, such as between 115-180 kDa.*

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

Western blot	Routinely Tested
Immunoprecipitation	Tested During Development

BD Biosciences

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Recommended Assay Procedure:

Western blot: Please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

For western blots, an extended transfer from a low percentage gel to the blot is necessary because the DNA-PK protein is so large. For example, a 4-12 hour transfer at 4°C may be needed for a 7.5% gel. HBL-100 human breast carcinoma cells (ATCC HTB-124) have been reported to be helpful as a positive control.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Kirchgessner CU, Patil CK, Evans JW, et al. DNA-dependent kinase (p350) as a candidate gene for the murine SCID defect. *Science.* 1995; 267(5201):1178-1183.(Biology)

Lees-Miller SP, Godbout R, Chan DW, et al. Absence of p350 subunit of DNA-activated protein kinase from a radiosensitive human cell line. *Science.* 1995; 267(5201):1183-1185.(Biology)

Peterson SR, Kurimasa A, Oshimura M, Dynan WS, Bradbury EM, Chen DJ. Loss of the catalytic subunit of the DNA-dependent protein kinase in DNA double-strand-break-repair mutant mammalian cells. *Proc Natl Acad Sci U S A.* 1995; 92(8):3171-3174.(Biology)