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Datasheet for 600-401-494

DNA PKcs phosphoT2609 Antibody

Overview

Description:	Anti-DNA PKcs pT 2609 (RABBIT) Antibody - 600-401-494
Item No.:	600-401-494
Size:	100 µg
Applications:	ELISA, WB, IHC
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	DNA dependent Protein Kinase (also called DNAPK, DNPK1, HYRC1, Protein Kinase DNA Activated Catalytic Polypeptide, XRCC7 and P460) consists of the 460 kDa DNA PKcs and a heterodimeric regulatory complex comprised of p70 Ku and p80 Ku (Ku autoantigen). DNA PKcs is a nuclear protein serine/threonine kinase present in a wide variety of eukaryotic species. DNA PKcs phosphorylates transcription factors, Sp1, Oct-1, p53 and SV40 large T antigen. DNA PKcs is involved in repairing double stranded DNA breaks. At the onset of apoptosis, DNA PKcs is rapidly inactivated by cleavage of the catalytic subunit into smaller polypeptides. Proteolysis of DNA PKcs is inhibited by the cysteine protease inhibitors iodoacetamide and N-ethylmaleimide. Alternative splicing can occur for this protein to produce at least two isoforms. Rabbit Anti-DNA PKcs pT2609 Antibody is useful for researchers interested in DNA damage.
Synonyms:	rabbit anti-DNA PKcs pT2609 Antibody, DNA dependent protein kinase catalytic subunit antibody, Protein Kinase DNA-Activated Catalytic Polypeptide, DNA PK catalytic subunit antibody, DNA-PK antibody, DNPK-1 antibody, DNAPK catalytic subunit antibody, DNPK 1 antibody, DNPK1 antibody, HYRC1 antibody, HYRC, P460, Hyper-Radiosensitivity Of Murine Scid Mutation Complementing 1, IMD26, XRCC7, P350
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	PRKDC

Reactivity:	Human
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	Rabbit Anti-DNA PKcs pT2609 affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic phospho-peptide corresponding to amino acids surrounding Thr 2609 of human DNA PKcs.
Purity/Specificity:	DNA PKcs pT2609 antibody is directed against the phosphorylated form of human DNA PKcs at the pT2609 residue. The product was affinity purified from monospecific antiserum by immunoaffinity purification. Antiserum was first purified against the phosphorylated form of the immunizing peptide. The resultant affinity purified antibody was then cross-adsorbed against the non-phosphorylated form of the immunizing peptide. This phospho-specific polyclonal antibody reacts with phosphorylated pT2609 of human DNA PKcs. Reactivity with non-phosphorylated human DNA PKcs is minimal. A BLAST analysis was used to suggest reactivity with this protein from human and chimpanzee based on 100% homology for the immunogen sequence. However, cross-reactivity is expected with mouse, rat, dog, chicken and horse DNA PKcs based on a high degree of homology to the immunogen sequence. Cross-reactivity with DNA PKcs homologues from other sources has not been determined.
Relevant Links:	• UniProtKB - P78527 • NCBI - 13654237 • GeneID - 5591

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	IHC (Based on references)
Application Note:	Anti-DNA PKcs pT2609 antibody has been tested for use in ELISA, western blot, and IP. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 460 kDa in size corresponding to DNA PKcs by western blotting in the appropriate cell lysate or extract. Alternate splice variants have been described for this protein. Best western blotting results are seen when IP is performed prior to detection. This antibody detects an inducible signal at the correct height after DNA damage. However, the antibody may also detect an IR-inducible signal in cells lacking DNA PKcs (MO59J cells) at the same size as DNA PKcs if western blotting is performed directly. We believe that this additional band is 53BP1 that runs at the same size as DNA PKcs and is also phosphorylated in an IR-dependent manner. Similar results were seen with another phospho DNA PKcs antibody indicating that this result is general to antibodies to this phospho site, rather than specific to this antibody.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:300,000

IP:	User Optimized
WB:	1:500 - 1:2,000

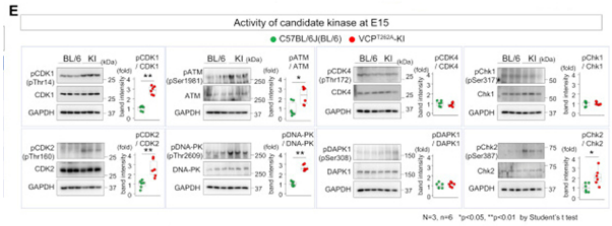
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/ml by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

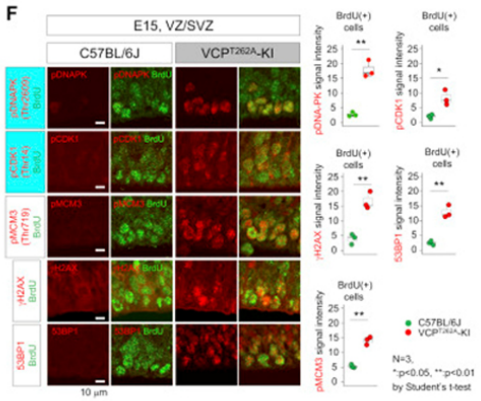
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

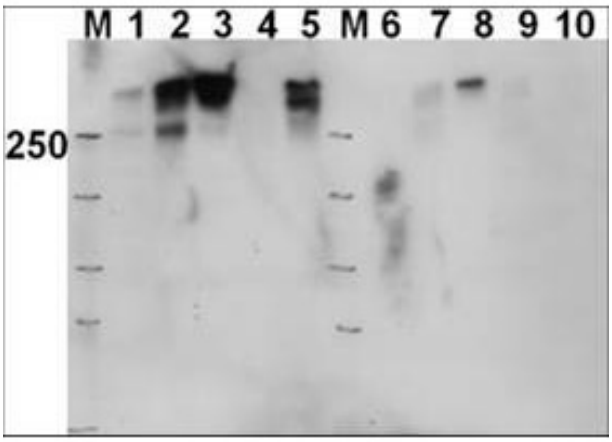
Images



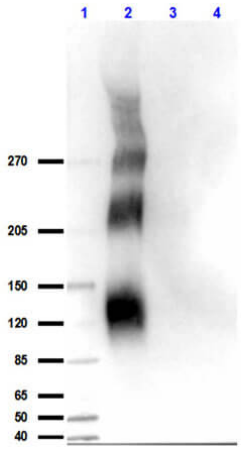
Western Blot
(E) Western blots revealed distinct levels of various DNA damage response kinases in developmental cortex at E15. Right graphs show quantitative analyses of three C57BL/6J and three VCPT262A-KI mice. Figure 3.
PMID: 34130995



Western Blot
(F) Immunohistochemistry of the active form of DNA-PK, the active form of CDK1, phosphorylated MCM3, and DNA damage markers (γH2AX and 53BP1). The active form of DNA-PK, phospho-MCM3 and DNA damage markers are detected in apical NSCs, whereas the active forms of CDK1 and DNA damage markers were detected in basal NSCs at E15. The right graph shows average signal intensities of 30 cells per field from each mouse (N = 3). Figure 3.
PMID: 34130995



Western Blot
Western blot using Rockland's Affinity Purified anti-DNAPKcs antibody shows detection of a 460 kDa band corresponding to human DNAPKcs in various preparations. Lane 1: Fus1 untreated, Lane 2: Fus1 IR (20Gy, 4h), Lane 3: Fus1 DNAPK inhibitor + IR, Lane 4: MO59J (DNAPK-) untreated, Lane 5: MO59J IR, Lane 6: Fus1 untreated, Lane 7: Fus1 IR (20Gy, 4h), Lane 8: Fus1 DNAPK inhibitor + IR, Lane 9: MO59J untreated, Lane 10: MO59J IR. Lanes 1-5 are nuclear extract whereas lanes 6-10 are whole cell lysates. MO59J is a cell line that lacks DNA-PKcs. FUS1 is the matched cell line complemented with a chromosomal fragment containing the DNA-PKcs gene. Approximately 20 µg of lysate was run on SDS-PAGE and transferred onto nitrocellulose, followed by reaction with a 1:1,000 dilution of anti-DNAPKcs antibody. Detection occurred using a 1:5,000 dilution of HRP-labeled Goat anti-Rabbit IgG for 1 hour at room temperature. A chemiluminescence system was used for signal detection (Roche) using a 1 min exposure time.



Western Blot
Western Blot results of Rabbit Anti-DNA Pkcs pT609 Antibody. Lane 1: Molecular Weight Ladder. Lane 2: DNA Pkc phosphor control. Lane 3: DNA Pkc control. Lane 4: BSA. Load: 10µg. Primary Antibody: Rabbit Anti-DNA Pkcs pT609 at 1µg/mL overnight at 4°C. Secondary Antibody: Goat anti-Rabbit HRP (p/n 611-103-122) at 1:70,000 for 30min at RT. Blocking: BlockOut (p/n MB-073) for 30 min at RT.

References

- Homma H et al. DNA damage in embryonic neural stem cell determines FTLDs' fate via early-stage neuronal necrosis. *Life Sci Alliance.* (2021)

Disclaimer

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