abcam

Product datasheet

Anti-DNA PKcs (phospho S2056) antibody [EPR5670] - BSA and Azide free ab174576

Recombinant RabMAb

2 References 7 Images

Overview

Product name	Anti-DNA PKcs (phospho S2056) antibody [EPR5670] - BSA and Azide free
Description	Rabbit monoclonal [EPR5670] to DNA PKcs (phospho S2056) - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, WB, ELISA, ICC/IF, ChIC/CUT&RUN-seq
Species reactivity	**Reacts with:** Human **Does not react with:** Mouse, Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Jurkat whole cell lysate (**ab7899**). IHC-P: Human lung carcinoma and brain tissues. ICC/IF: Jurkat cells. ChIC/CUT&RUN-Seq: U2OS cells.
General notes	ab174576 is the carrier-free version of **ab124918**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Purification notes	Protein-A purification via MabSelect SuRe
Clonality	Monoclonal
Clone number	EPR5670
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab174576 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

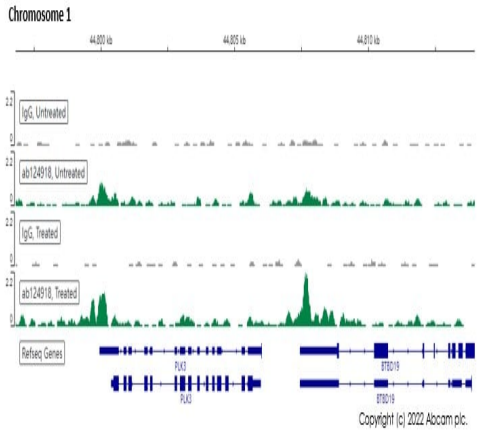
Application	Abreviews	Notes
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
WB		Use at an assay dependent concentration. Predicted molecular weight: 469 kDa.
ELISA		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.

Target

Function Serine/threonine-protein kinase that acts as a molecular sensor for DNA damage. Involved in DNA nonhomologous end joining (NHEJ) required for double-strand break (DSB) repair and V(D)J recombination. Must be bound to DNA to express its catalytic properties. Promotes processing of hairpin DNA structures in V(D)J recombination by activation of the hairpin endonuclease artemis (DCLRE1C). The assembly of the DNA-PK complex at DNA ends is also required for the NHEJ ligation step. Required to protect and align broken ends of DNA. May also act as a scaffold protein to aid the localization of DNA repair proteins to the site of damage. Found at the ends of chromosomes, suggesting a further role in the maintenance of telomeric

	stability and the prevention of chromosomal end fusion. Also involved in modulation of transcription. Recognizes the substrate consensus sequence [ST]-Q. Phosphorylates 'Ser-139' of histone variant H2AX/H2AFX, thereby regulating DNA damage response mechanism. Phosphorylates DCLRE1C, c-Abl/ABL1, histone H1, HSPCA, c-jun/JUN, p53/TP53, PARP1, POU2F1, DHX9, SRF, XRCC1, XRCC1, XRCC4, XRCC5, XRCC6, WRN, MYC and RFA2. Can phosphorylate C1D not only in the presence of linear DNA but also in the presence of supercoiled DNA. Ability to phosphorylate p53/TP53 in the presence of supercoiled DNA is dependent on C1D.
Sequence similarities	Belongs to the PI3/PI4-kinase family. Contains 1 FAT domain. Contains 1 FATC domain. Contains 2 HEAT repeats. Contains 1 PI3K/PI4K domain. Contains 3 TPR repeats.
Post-translational modifications	Phosphorylated upon DNA damage, probably by ATM or ATR. Autophosphorylated on Thr-2609, Thr-2638 and Thr-2647. Thr-2609 is a DNA damage-inducible phosphorylation site (inducible with ionizing radiation, IR). Autophosphorylation induces a conformational change that leads to remodeling of the DNA-PK complex, requisite for efficient end processing and DNA repair. S-nitrosylated by GAPDH.
Cellular localization	Nucleus.

Images



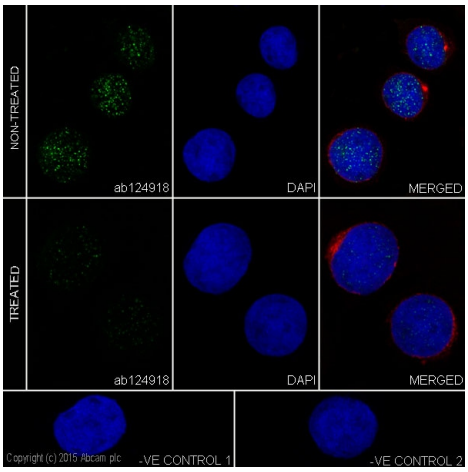
ChIC/CUT&RUN sequencing - Anti-DNA PKcs (phospho S2056) antibody [EPR5670] - BSA and Azide free (ab174576)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5 x 10^5 U2OS cells treated with Etoposide (10µM 20h) and 5 µg of **ab124918** [EPR5670]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown.

Additional screenshots of mapped reads can be downloaded **here**.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124918**).



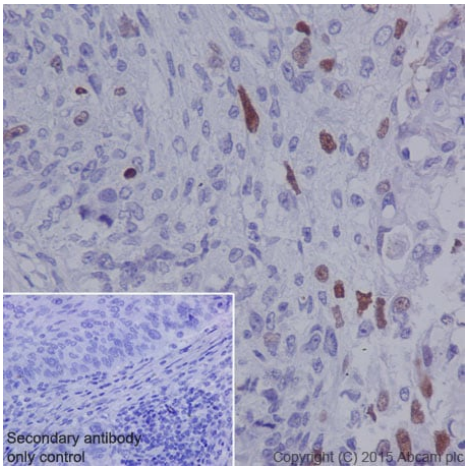
Immunocytochemistry/ Immunofluorescence - Anti-DNA PKcs (phospho S2056) antibody [EPR5670] - BSA and Azide free (ab174576)

Immunocytochemistry/Immunofluorescence analysis of Jurkat cells (untreated and treated with Alkaline Phosphatase) labelling DNA PKcs (phospho S2056) with purified **ab124918** at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.

Control 1: primary antibody (1/500) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).

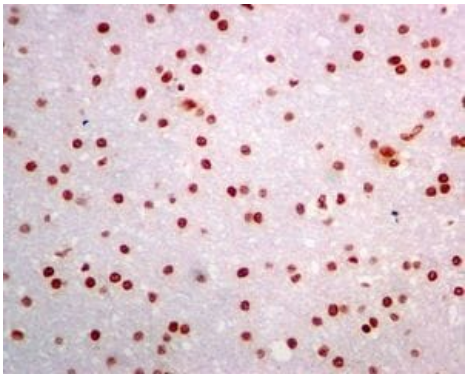
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124918**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-DNA PKcs (phospho S2056) antibody [EPR5670] - BSA and Azide free (ab174576)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung carcinoma tissue labelling DNA PKcs (phospho S2056) with purified **ab124918** at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124918**).

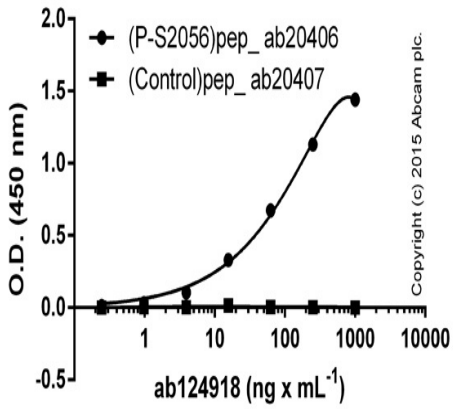


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-DNA PKcs (phospho S2056) antibody [EPR5670] - BSA and Azide free (ab174576)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human brain tissue labelling DNA PKcs (phospho S2056) with unpurified **ab124918** at a dilution of 1/100.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124918**).

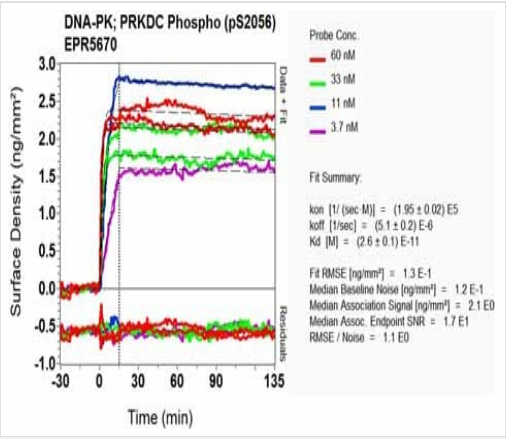
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



ELISA - Anti-DNA PKcs (phospho S2056) antibody [EPR5670] - BSA and Azide free (ab174576)

Serially diluted **ab124918** was bound to immobilised phospho- or control peptides (1 microgram per mL). The antibody was detected by HRP-labelled goat anti-rabbit IgG (**ab97080**; diluted 50000 times) and signal was developed with TMB substrate.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124918**).



OI-RD Scanning - Anti-DNA PKcs (phospho S2056) antibody [EPR5670] - BSA and Azide free (ab174576)

Anti-DNA PKcs (phospho S2056) antibody [EPR5670] - BSA and Azide free (ab174576)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124918**).

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