abcam

Product datasheet

Anti-DNA PKcs antibody [18-2] ab44815

KO VALIDATED

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Overview

Product name	Anti-DNA PKcs antibody [18-2]
Description	Mouse monoclonal [18-2] to DNA PKcs
Host species	Mouse
Tested applications	**Suitable for:** ICC/IF, WB
Species reactivity	**Reacts with:** Human
Immunogen	Other Immunogen Type corresponding to Human DNA PKcs aa 1-2713. Immunogen is purified from HeLa cells. Database link: **P78527**
Epitope	amino acids 1-2713
Positive control	WB: Wildtype HAP1, SHSY5Y and K562 cell lysates. ICC/IF: Wildtype HAP1 cells.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.1% Sodium azide Constituent: PBS
Purity	Protein G purified
Clonality	Monoclonal
Clone number	18-2

Isotype IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab44815 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
ICC/IF		1/100.
WB	★★★★★ (1)	1/200. Predicted molecular weight: 469 kDa.

Target

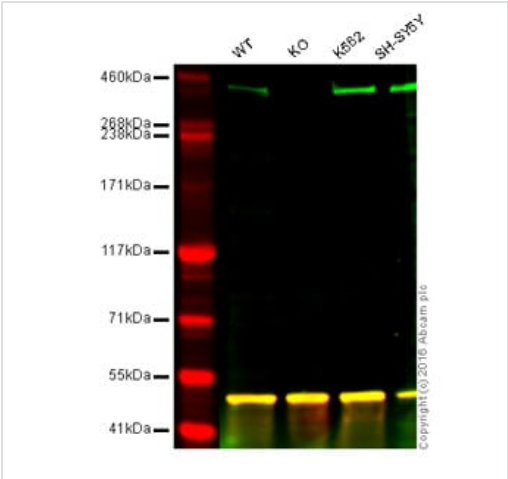
Function Serine/threonine-protein kinase that acts as a molecular sensor for DNA damage. Involved in DNA nonhomologous end joining (NHEJ) required for double-strand break (DSB) repair and V(D)J recombination. Must be bound to DNA to express its catalytic properties. Promotes processing of hairpin DNA 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.

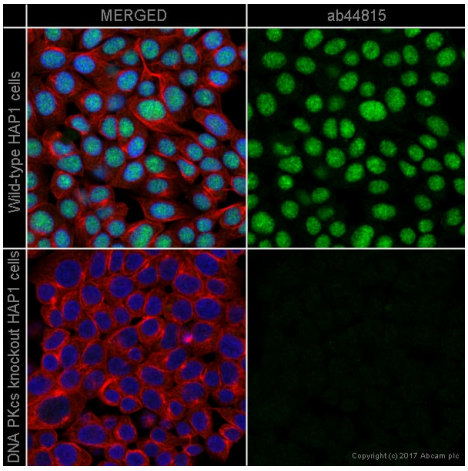
Images



Western blot - Anti-DNA PKcs antibody [18-2] (ab44815)

Lane 1: Wild-type HAP1 cell lysate (40 µg)
Lane 2: DNA PKcs knockout HAP1 cell lysate (40 µg)
Lane 3: K562 cell lysate (20 µg)
Lane 4: SHSY5Y cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab44815 observed at 460 kDa. Red - loading control, **ab18251**, observed at 52 kDa.

ab44815 was shown to specifically react with DNA PKcs in iwld-type HAP1 cells. No band was observed when DNA PKcs knockout samples were examined. Wild-type and DNA PKcs knockout samples were subjected to SDS-PAGE. Ab44815 and **ab18251** (loading control to alpha tubulin) were diluted at 1/200 and 1/10,000 dilution respectively and incubated overnight at 4C. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed **ab216772** and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed **ab216777** secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-DNA PKcs antibody [18-2] (ab44815)

ab44815 staining DNA PKcs in wild-type HAP1 cells (top panel) and PRKDC knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab44815 at 1/100 dilution and **ab202272** at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Mouse IgG (Alexa Fluor® 488) (**ab150117**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

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