abcam

Product datasheet

Anti-DNA PKcs (phospho S2056) antibody ab18192

敲除验证

14 Abreviews 184 References 6 图像

概述

产品名称	Anti-DNA PKcs (phospho S2056)抗体
描述	兔多克隆抗体to DNA PKcs (phospho S2056)
宿主	Rabbit
特异性	From Jan 2024, QC testing of replenishment batches of this polyclonal changed. All tested and expected application and reactive species combinations are still covered by our Abcam product promise. However, we no longer test all applications. For more information on a specific batch, please contact our Scientific Support who will be happy to help. This antibody specifically recognizes a band at ~460 kDa in HeLa cells that have been treated with ionizing radiation, that is not detected in untreated cells. This band can also be competed away by the immunizing modified peptide, but not the unmodified peptide containing the same amino acid sequence.All batches of this antibody are screened in ELISA and show high titres against the immunising peptide. Reactivity with the unmodified DNA PKcs peptide is minimal.We now predict that this antibody will cross react with mouse DNA PKcs, as the mouse sequence has been more extensively reviewed on uniprot (P97313), now indicating that mouse DNA PKcs S2053, which corresponds to human S2056, is also phosphorylated. We welcome any feedback from researchers who have used this antibody with mouse samples.
经测试应用	**适用于:** WB, ELISA, ICC/IF
种属反应性	**与反应:** Human **预测可用于:** Mouse
免疫原	**This product was produced with the following immunogens:** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	ICC/IF: HeLa UV cells.
常规说明	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

性能

形式 Liquid

存放说明 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.

存储溶液 pH: 7.40
Preservative: 0.02% Sodium azide
Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

纯度 Immunogen affinity purified

克隆 多克隆

同种型 IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab18192于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★★★ (4)	Use a concentration of 1 µg/ml. Detects a band of approximately 460 kDa (predicted molecular weight: 460 kDa).
ELISA		Use at an assay dependent concentration. Direct ELISA with serially diluted ab18192 (0.2-1000 ng x mL^{-1}), bound to immobilised phospho or control peptides (1 µg x mL^{-1}).
ICC/IF	★★★★★ (6)	Use a concentration of 1 - 5 µg/ml.

靶标

功能 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.

序列相似性

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.

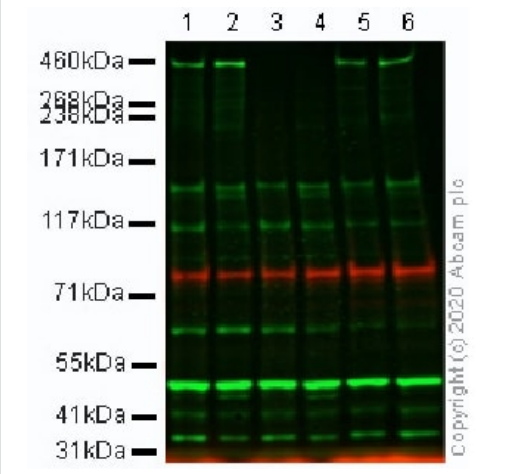
翻译后修饰

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.

细胞定位

Nucleus.

图片



Western blot - Anti-DNA PKcs (phospho S2056) antibody (ab18192)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: HAP1 Parental Camptothecin (1um 1hr) whole cell lysate (20 µg)
Lane 3: HAP1 PRKDC KO Untreated whole cell lysate (20 µg)
Lane 4: HAP1 PRKDC KO camptothecin (1um 1hr) whole cell lysate (20ug)

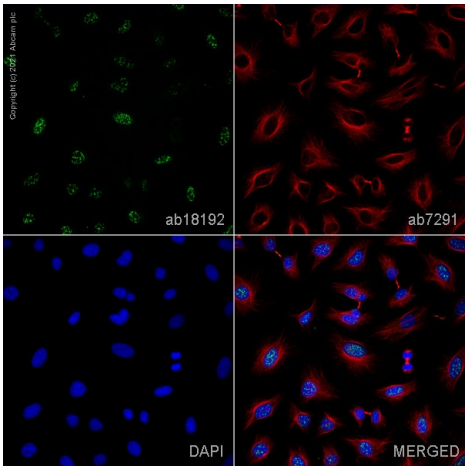
Lane 5: SHSY-5Y untreated whole cell lysate (20ug)

Lane 6: SHSY-5Y Camptothecin treated (1uM, 1hr) whole tissue lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab18192 observed at 450 kDa. Red - loading control, **ab6301**, observed at 85 kDa.

ab18192 was shown to specifically react with DNA PKcs (phospho S2056) in wild-type HAP1 cells along with additional cross reactive bands. Uplift was also seen with Camptothecin treatment and no bands were observed when DNA PKcs (phospho S2056) knockout cellsd. Wild-type and DNA PKcs (phospho S2056) knockout samples were subjected to SDS-PAGE. ab18192 and **ab6301** (Mouse anti-Beta Catenin loading control) were incubated overnight at 4°C at 1 µg/ml and 1/10,000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW)

preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.

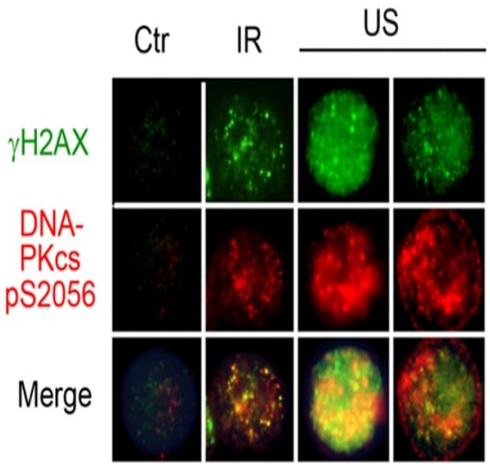


Immunocytochemistry/ Immunofluorescence - Anti-DNA PKcs (phospho S2056) antibody (ab18192)

ab18192 staining DNA PKcs (phospho S2056) in HeLa UV cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-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 overnight at 4°C with ab18192 at 1µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

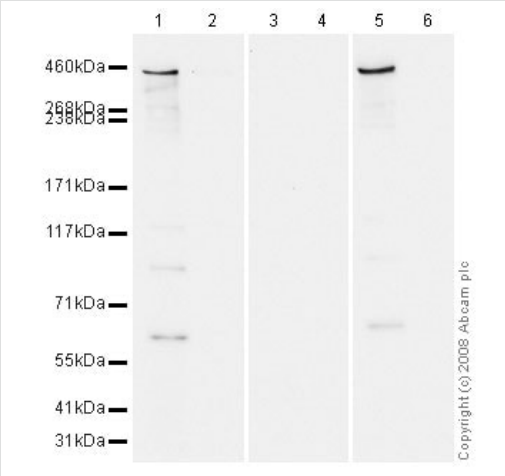


Immunocytochemistry/ Immunofluorescence - Anti-DNA PKcs (phospho S2056) antibody (ab18192)

Furusawa Y et al. DNA double-strand breaks induced by cavitational mechanical effects of ultrasound in cancer cell lines. PLoS One 7:e29012 (2012). Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

U937 (Human histiocytic lymphoma cell line) cells were paraformaldehyde-fixed. Control and treated cells were permeabilized/blocked with 2% BSA/0.05% Triton X-100/Tris-buffered saline, and immunostained for DNA PKcs (phospho S2056) (Red) for 2 h with ab18192 at 1/600 dilution.

Green staining shows γH2AX. IR = Ionzing radiation. US = Ultrasound.



Western blot - Anti-DNA PKcs (phospho S2056) antibody (ab18192)

All lanes : Anti-DNA PKcs (phospho S2056) antibody (ab18192) at 1 µg/ml

Lane 1 : Gamma Irradiated HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : Untreated HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 3 : Gamma Irradiated HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate with Human DNA PKcs (phospho S2056) peptide (**ab20406**) at 1 µg/ml
Lane 4 : Untreated HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate with Human DNA PKcs (phospho S2056) peptide (**ab20406**) at 1 µg/ml
Lane 5 : Gamma Irradiated HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate with Human DNA PKcs peptide (**ab20407**) at 1 µg/ml
Lane 6 : Untreated HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate with Human DNA PKcs peptide (**ab20407**) at 1 µg/ml

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Alexa Fluor Goat polyclonal to Rabbit IgG (700) at 1/10000 dilution

Developed using the ECL technique.

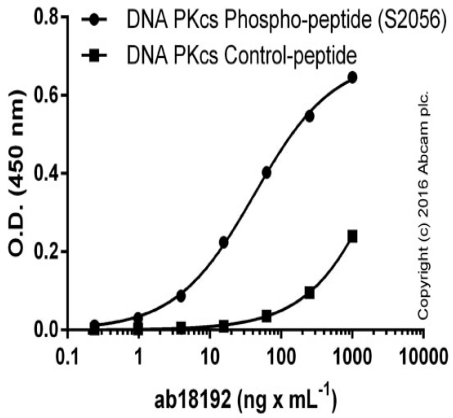
Performed under reducing conditions.

Predicted band size: 460 kDa
Observed band size: 460 kDa
Additional bands at: 270 kDa (possible cleavage fragment), 270 kDa (possible cross reactivity)

ab18192 specifically recognizes a band at ~460 kDa corresponding to DNA PKcs in HeLa cells that have been treated with ionizing radiation (lane 1). This band is not detected in

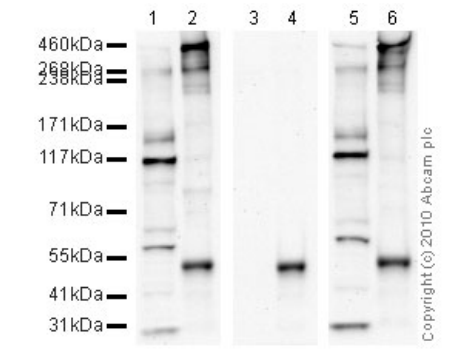
untreated cells (lane 2). The activity of the antibody is quenched by the addition of the immunizing (modified) peptide, **ab20406** (lanes 3) but not the unmodified peptide, **ab20407** (lane 5).

For the **ab13823** irradiated HeLa cell lysate, the 4 hour post-treatment extract was used.



ELISA - Anti-DNA PKcs (phospho S2056) antibody (ab18192)

Serially diluted ab18192 was bound to immobilised DNA PKcs phospho peptide (2052 - 2062; P-S2056) or DNA PKcs control peptide (2052 - 2062; both at 1 microgram x mL^{-1}). The antibody was detected by HRP-labelled goat anti-rabbit IgG (**ab97080**; diluted 50000 times) and signal was developed with TMB substrate.



Western blot - Anti-DNA PKcs (phospho S2056) antibody (ab18192)

All lanes : Anti-DNA PKcs (phospho S2056) antibody (ab18192) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : Hela Whole Cell Lysate - Bleomycin Treated (20U/ml)
Lane 3 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with Human DNA PKcs (phospho S2056) peptide (**ab20406**) at 1 µg/ml
Lane 4 : Hela Whole Cell Lysate - Bleomycin Treated (20U/ml) with Human DNA PKcs (phospho S2056) peptide (**ab20406**) at 1 µg/ml
Lane 5 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with Human DNA PKcs peptide (**ab20407**) at 1 µg/ml
Lane 6 : Hela Whole Cell Lysate - Bleomycin Treated (20U/ml) with Human DNA PKcs peptide (**ab20407**) at 1 µg/ml

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

Predicted band size: 460 kDa
Observed band size: 460 kDa
Additional bands at: 117 kDa, 150 kDa, 270 kDa, 50 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 15 minutes

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