abcam

Product datasheet

Anti-DNA PKcs antibody [Y393] ab32566

敲除验证 重组 RabMAb

13 Abreviews 36 References 12 图像

概述

产品名称	Anti-DNA PKcs抗体[Y393]
描述	兔单克隆抗体[Y393] to DNA PKcs
宿主	Rabbit
特异性	This antibody is specific for DNA PKcs. It may also detect the splice isoform 2. Mouse and rat species are recommended based on WB results, we do not guarantee IHC-P for mouse and rat.
经测试应用	**适用于:** ChIC/CUT&RUN-seq, WB, IHC-P, ICC/IF **不适用于:** Flow Cyt or IP
种属反应性	**与反应:** Human **预测可用于:** Mouse, Rat, Armenian hamster
免疫原	Synthetic peptide within Human DNA PKcs aa 4050 to the C-terminus (C terminal). The exact sequence is proprietary. Database link: **P78527**
阳性对照	WB: K562, MOLT4, SH-SY5Y, HeLa and Wild-type HAP1 cell lysate; Wild-type A549 cell lysate; HDLM-2 cell lysate. ICC/IF: Hela cells. IHC-P: Human breast carcinoma tissue slides, human tonsil tissue. ChIC/CUT&RUN-Seq: U2OS cells.
常规说明	Mouse and rat samples are recommended based on WB results. 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**.

性能

形式	Liquid

存放说明	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	Y393
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab32566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
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5 µg
WB	★★★★★ **(10)**	1/1000 - 1/10000. Detects a band of approximately 460 kDa (predicted molecular weight: 469 kDa). **For unpurified, use 1/1000 - 1/2000.**
IHC-P		1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. **For unpurified, use 1/5.**
ICC/IF	★★★★★ **(2)**	1/100. **For unpurified, use 1/10.**

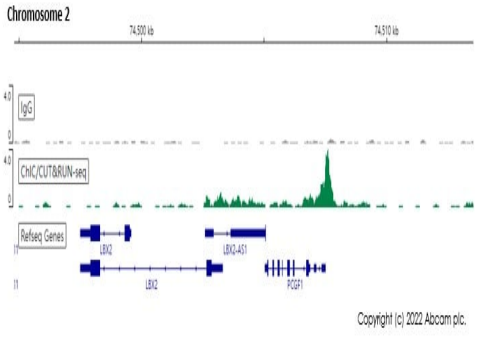
应用说明 Is unsuitable for Flow Cyt or IP.

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

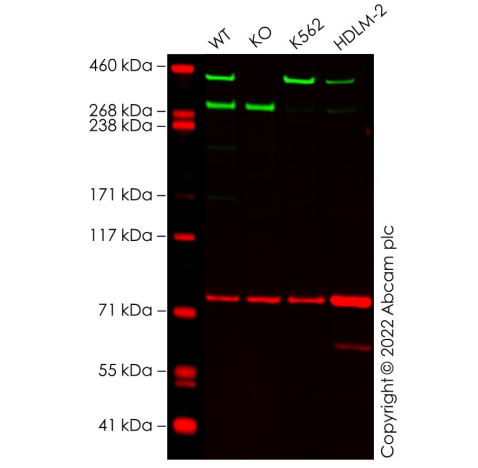


ChIC/CUT&RUN sequencing - Anti-DNA PKcs antibody [Y393] (ab32566)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2 x 10^5 U2OS cells and 5 µg of ab ab32566 [Y393]. 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.



Western blot - Anti-DNA PKcs antibody [Y393] (ab32566)

All lanes : Anti-DNA PKcs antibody [Y393] (ab32566) at 1/1000 dilution

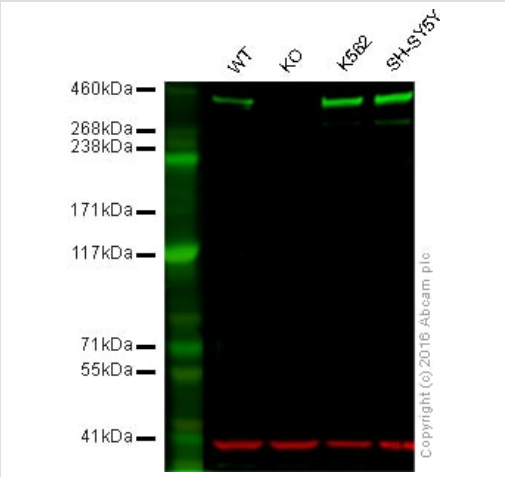
Lane 1 : Wild-type A549 cell lysate
Lane 2 : PRKDC knockout A549 cell lysate
Lane 3 : K562 cell lysate
Lane 4 : HDLM-2 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 469 kDa
Observed band size: 450 kDa

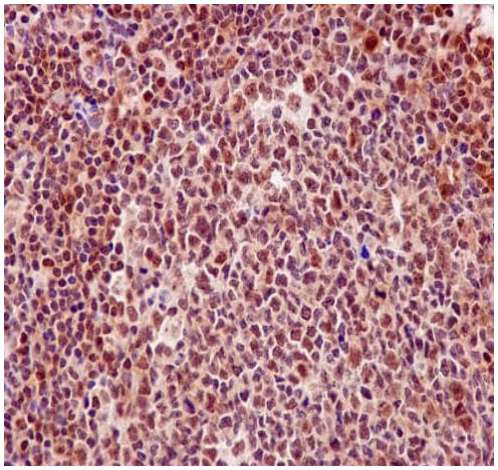
False colour image of Western blot: Anti-DNA PKcs antibody [Y393] staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (**ab238078**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32566 was shown to bind specifically to DNA PKcs. A band was observed at 450 kDa in wild-type A549 cell lysates with no signal observed at this size in PRKDC knockout cell line. To generate this image, wild-type and PRKDC knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-DNA PKcs antibody [Y393] (ab32566)

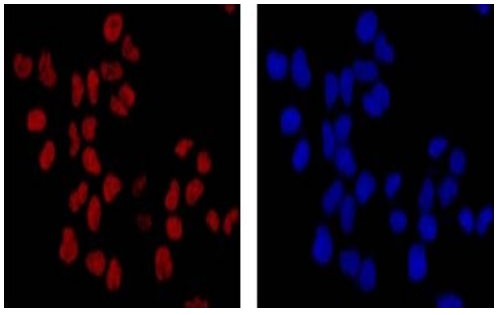
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: DNA PKcs knockout HAP1 cell lysate (20 µg)
Lane 3: K562 cell lysate (20 µg)
Lane 4: SH-SY5Y cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab32566 observed at 470 kDa. Red - loading control, **ab7291**, observed at 52 kDa.

ab32566 was shown to specifically react with DNA PKCs in wild-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. ab32566 and **ab7291** (loading control to alpha tubulin) were diluted 1/1000 and 1/10,000 and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.



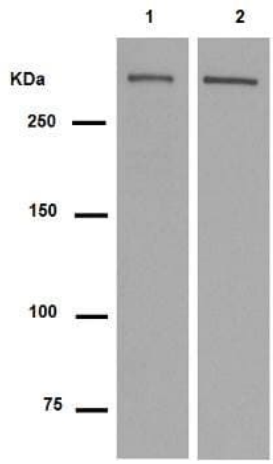
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-DNA PKcs antibody [Y393] (ab32566)

Immunohistochemical staining of paraffin embedded human tonsil with purified ab32566 at a working dilution of 1 in 50. The secondary antibody used is a HRP polymer for rabbit IgG. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0.



Immunocytochemistry/ Immunofluorescence - Anti-DNA PKcs antibody [Y393] (ab32566)

Immunofluorescent staining of HeLa cells (fixed with 4% PFA) with purified ab32566 at a dilution of 1/100. An Alexa Fluor® 555 goat anti-rabbit antibody was used as the secondary at a dilution of 1/200. The panel on the right shows the DAPI counter-staining.



Western blot - Anti-DNA PKcs antibody [Y393] (ab32566)

All lanes : Anti-DNA PKcs antibody [Y393] (ab32566) at 1/7300 dilution (purified)

Lane 1 : MOLT4 cell lysate
Lane 2 : HeLa cell lysate

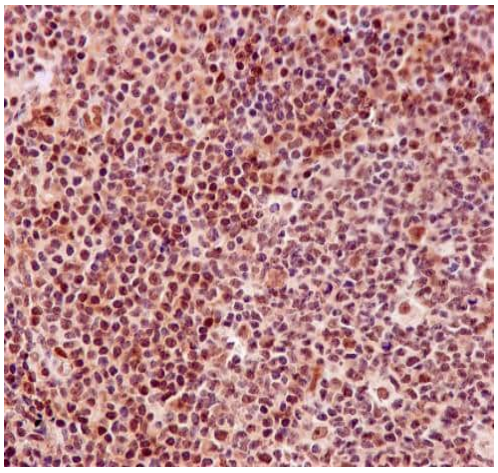
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 469 kDa
Observed band size: 460 kDa

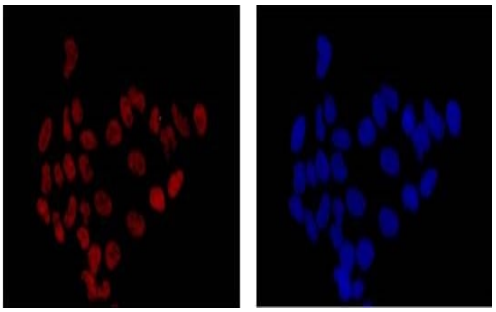
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



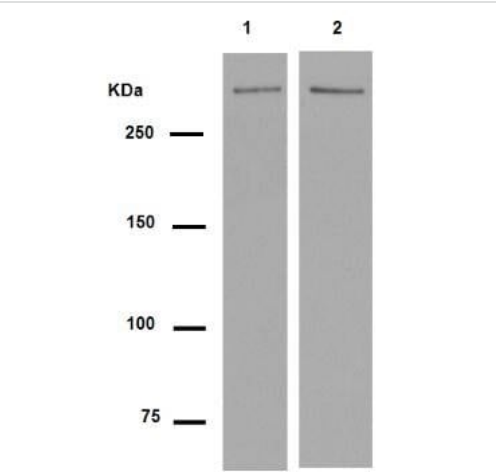
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-DNA PKcs antibody [Y393] (ab32566)

Immunohistochemical staining of paraffin embedded human tonsil with unpurified ab32566 at a working dilution of 1 in 5. The secondary antibody used is a HRP polymer for rabbit IgG. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0.



Immunocytochemistry/ Immunofluorescence - Anti-DNA PKcs antibody [Y393] (ab32566)

Immunofluorescent staining of HeLa cells (fixed with 4% PFA) with unpurified ab32566 at a dilution of 1/10. An Alexa Fluor® 555 goat anti-rabbit antibody was used as the secondary at a dilution of 1/200. The panel on the right shows the DAPI counter-staining.



Western blot - Anti-DNA PKcs antibody [Y393] (ab32566)

All lanes : Anti-DNA PKcs antibody [Y393] (ab32566) at 1/1000 dilution (unpurified)

Lane 1 : MOLT4 cell lysate
Lane 2 : HeLa cell lysate

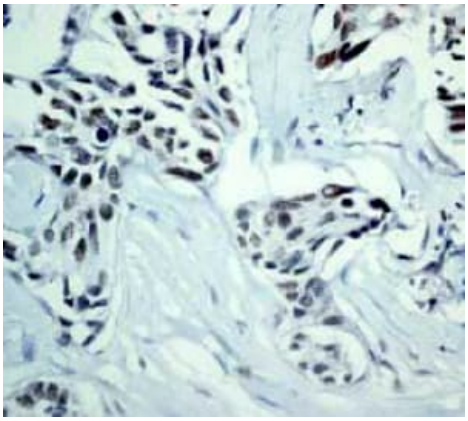
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 469 kDa
Observed band size: 460 kDa

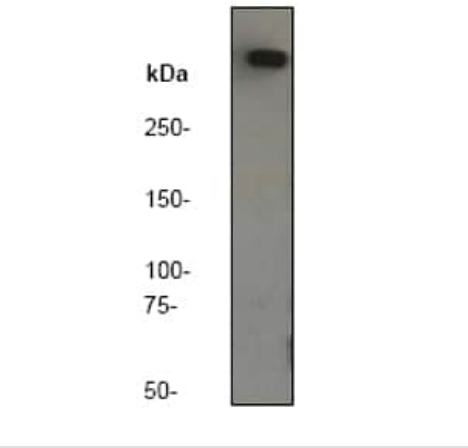
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-DNA PKcs antibody [Y393] (ab32566)

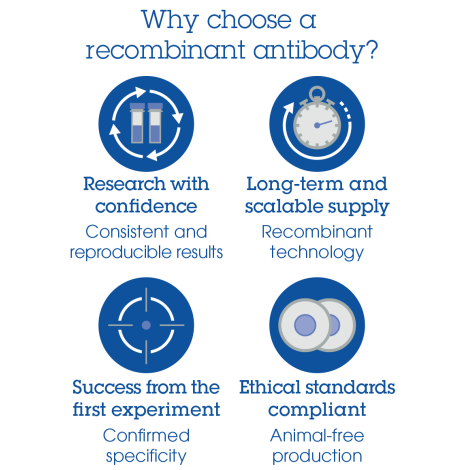
Unpurified ab32566, at a 1/50 dilution, staining DNA PKcs in paraffin embedded human breast carcinoma tissue by immunohistochemistry.



Western blot - Anti-DNA PKcs antibody [Y393] (ab32566)

Anti-DNA PKcs antibody [Y393] (ab32566) at 1/1000 dilution (unpurified) + K562 cell lysate.

Predicted band size: 469 kDa
Observed band size: 460 kDa



Anti-DNA PKcs antibody [Y393] (ab32566)

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