abcam

Product datasheet

Anti-DNA PKcs antibody [EPR392] ab133516

KO VALIDATED Recombinant RabMAb

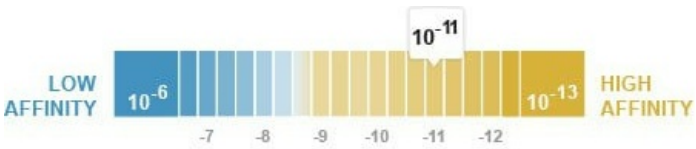
1 References 7 Images

Overview

Product name	Anti-DNA PKcs antibody [EPR392]
Description	Rabbit monoclonal [EPR392] to DNA PKcs
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF **Unsuitable for:** Flow Cyt,IHC-P or IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human DNA PKcs aa 50-150. The exact sequence is proprietary. Database link: **P78527**
Positive control	WB: K562, Molt4, MCF7, SH-SY5Y, 293T and PC3 cell lysates. ICC/IF: K-562 cells
General notes	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 -20°C. Stable for 12 months at -20°C.
Dissociation constant (K_D)	K_D = 8.50 x 10^{-11} M



Learn more about K_D

Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR392
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab133516 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
WB		1/1000 - 1/10000. Detects a band of approximately 460 kDa (predicted molecular weight: 469 kDa).
ICC/IF		1/200. **For unpurified use at 1/500 - 1/1000.**

Application notes Is unsuitable for Flow Cyt,IHC-P or IP.

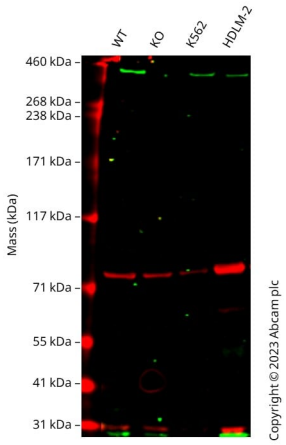
Target

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 [EPR392] (ab133516)

All lanes : Anti-DNA PKcs antibody [EPR392] (ab133516) at 1/2000 dilution

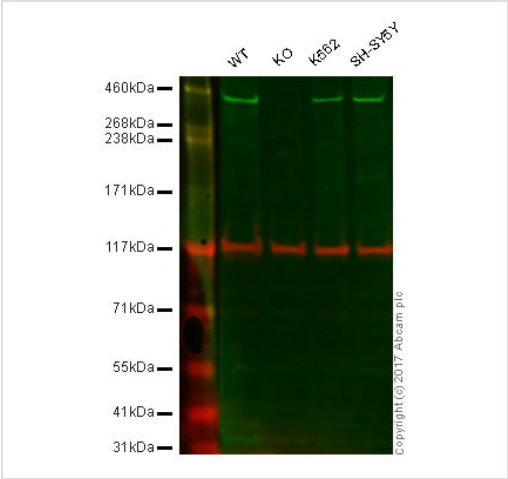
Lane 1 : Wild-type A549 cell lysate
Lane 2 : PRKDC knockout A549 cell lysate
Lane 3 : K562 cell lysate
Lane 4 : HDLM-2 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

Anti-PRKDC antibody [EPR392] (ab133516) staining at 1/2000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (**ab238078**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab133516 was shown to bind specifically to PRKDC. 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.

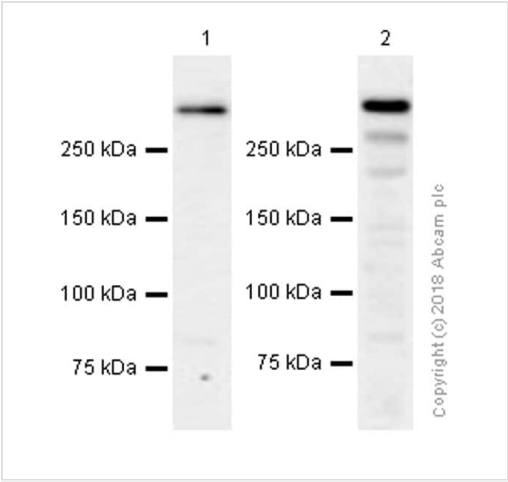


Western blot - Anti-DNA PKcs antibody [EPR392] (ab133516)

Lane 1: Wild type HAP1 whole cell lysate (40 µg)
Lane 2: DNA PKcs knockout HAP1 whole cell lysate (40 µg)
Lane 3: K562 whole cell lysate (20 µg)
Lane 4: SHSY5Y whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab133516 observed at 460 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab133516 was shown to specifically react with DNA PKcs when DNA PKcs knockout samples were used. Wild-type and DNA PKcs knockout samples were subjected to SDS-PAGE. Ab133516 and **ab18058** (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1000 dilution and 1/10000 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 1/10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-DNA PKcs antibody [EPR392] (ab133516)

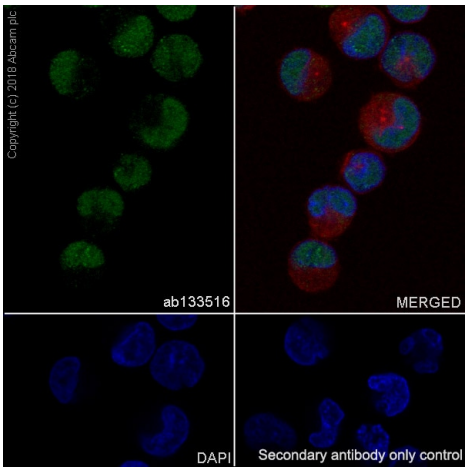
All lanes : Anti-DNA PKcs antibody [EPR392] (ab133516) at 1/5000 dilution (Purified)

Lane 1 : K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysates
Lane 2 : SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysates

Lysates/proteins at 15 µg per lane.

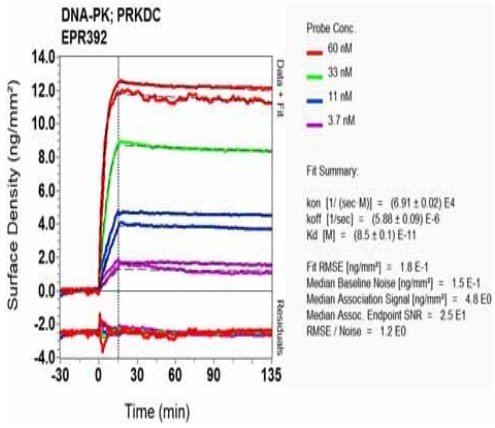
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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



Immunocytochemistry/ Immunofluorescence - Anti-DNA PKcs antibody [EPR392] (ab133516)

Immunocytochemistry/ Immunofluorescence analysis of K-562 (Human chronic myelogenous leukemia lymphoblast) cells labeling DNA PKcs with purified ab133516 at 1:200 dilution (9.3 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

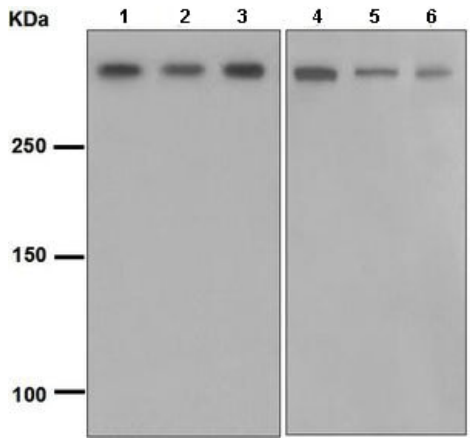


OI-RD Scanning - Anti-DNA PKcs antibody [EPR392] (ab133516)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D



Western blot - Anti-DNA PKcs antibody [EPR392] (ab133516)

All lanes : Anti-DNA PKcs antibody [EPR392] (ab133516) at 1/1000 dilution

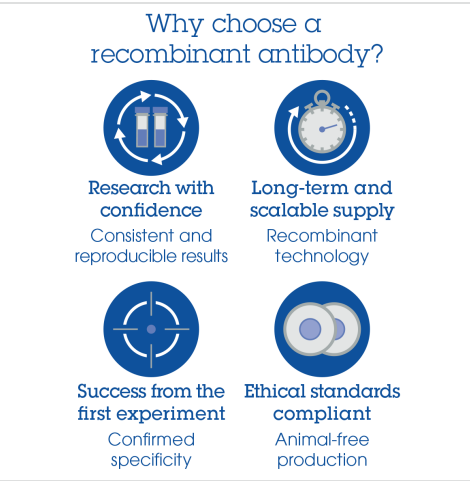
Lane 1 : K562 cell lysate
Lane 2 : Molt4 cell lysate
Lane 3 : MCF7 cell lysate
Lane 4 : SH-SY5Y cell lysate
Lane 5 : 293T cell lysate
Lane 6 : PC3 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat-anti-rabbit HRP at 1/2000 dilution

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



Anti-DNA PKcs antibody [EPR392] (ab133516)

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