abcam

Product datasheet

Anti-DNA Ligase IV/LIG4 antibody [EPR16531] ab193353





RabMAb



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Overview

Product name Anti-DNA Ligase IV/LIG4 antibody [EPR16531]

Description Rabbit monoclonal [EPR16531] to DNA Ligase IV/LIG4

Host species Rabbit

Tested applications Suitable for: IHC-P, ICC/IF, WB

Species reactivity Reacts with: Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa, Jurkat and Ramos cell lysate. IHC-P Human thymus tissue. ICC/IF: SH-SY5Y and wild-

type HAP1 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 $\mathsf{RabMAb}^{\texttt{®}}$ technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol (glycerin, glycerine), 59% PBS, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR16531

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab193353 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		1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use a concentration of 5 µg/ml.
WB	★★★ ☆☆ (4)	1/1000 - 1/2000. Predicted molecular weight: 103 kDa.

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Function Efficiently joins single-strand breaks in a double-stranded polydeoxynucleotide in an ATP-

dependent reaction. Involved in DNA non-homologous end joining (NHEJ) required for double-strand break repair and V(D)J recombination. The LIG4-XRCC4 complex is responsible for the NHEJ ligation step, and XRCC4 enhances the joining activity of LIG4. Binding of the LIG4-XRCC4 complex to DNA ends is dependent on the assembly of the DNA-dependent protein

kinase complex DNA-PK to these DNA ends.

Tissue specificity Testis, thymus, prostate and heart.

Involvement in disease LIG4 syndrome

Severe combined immunodeficiency autosomal recessive T-cell-negative/B-cell-negative/NK-cell-

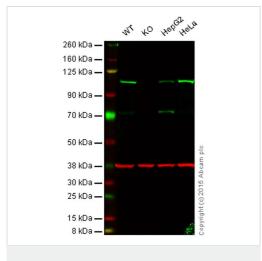
positive with sensitivity to ionizing radiation

Sequence similarities Belongs to the ATP-dependent DNA ligase family.

Contains 2 BRCT domains.

Cellular localization Nucleus.

Images



Western blot - Anti-DNA Ligase IV/LIG4 antibody [EPR16531] (ab193353)

WERCH APPI cells

WERCH APPI cells

WERCH APPI cells

Immunocytochemistry/ Immunofluorescence - Anti-DNA Ligase IV/LIG4 antibody [EPR16531] (ab193353)

Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: DNA Ligase IV/LIG4 knockout HAP1 cell lysate (20 µg)

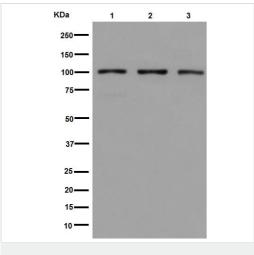
Lane 3: HepG2 cell lysate (20 µg)

Lane 4: HeLa cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab193353 observed at 103 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab193353 was shown to specifically react with DNA Ligase IV/LIG4 when DNA ligase IV knockout samples were used. Wild-type and DNA Ligase IV/LIG4 knockout samples were subjected to SDS-PAGE. ab193353 and ab8245 (loading control to GAPDH) were diluted 1/1000 and 1/2000 respectively 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 h at room temperature before imaging.

ab193353 staining DNA Ligase N/LIG4 in wild-type HAP1 cells (top panel) and LIG4 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 ab193353 at 5 μ g/ml dilution and ab195889 at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 μ g/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.



Western blot - Anti-DNA Ligase IV/LIG4 antibody [EPR16531] (ab193353)

All lanes : Anti-DNA Ligase IV/LIG4 antibody [EPR16531] (ab193353) at 1/1000 dilution

Lane 1 : HeLa cell lysate
Lane 2 : Jurkat cell lysate

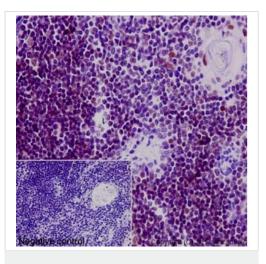
Lane 3: Ramos cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

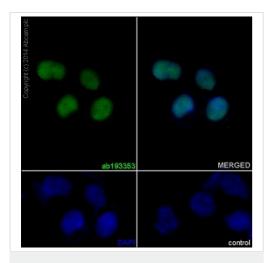
Predicted band size: 103 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-DNA Ligase IV/LIG4 antibody [EPR16531] (ab193353)

Immunohistochemical analysis of paraffin embedded Human thymus tissue sections labeling DNA Ligase IV/LIG4 using ab193353 at a 1/50 dilution. A ready to use HRP Polymer for Rabbit IgG was used as the secondary. Hematoxylin counterstain.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-DNA Ligase IV/LIG4 antibody [EPR16531] (ab193353)

Immunofluorescent analysis of 4% paraformaldehyde fixed SH-SY5Y cells labeling DNA Ligase IV/LIG4 using ab193353 at a 1/50 dilution. A Goat anti rabbit IgG (Alexa Fluor®488) (ab150077) was used as the secondary at a 1/400 dilution. Counterstain DAPI. Cells were permeabilized using 0.1% Triton X-100.



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