abcam

Product datasheet

Anti-Rad51 antibody [EPR4030(3)] ab133534

Recombinant RabMAb

★★★★ 15 Abreviews 111 References 14 Images

Overview

Product name Anti-Rad51 antibody [EPR4030(3)]

Description Rabbit monoclonal [EPR4030(3)] to Rad51

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra)

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: C6, NIH/3T3, 293T, Jurkat, HeLa, and K562 cell lysates and mouse spleen tissue lysate.

IHC-P: Human cervix carcinoma and testis tissues. ICC/IF: Jurkat cells. Flow Cyt (intra): HeLa

cells. IP: HEK293 cell lysates.

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**.

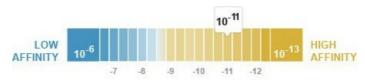
Properties

Form Liquid

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

Stable for 12 months at -20°C.

 $K_D = 9.20 \times 10^{-11} M$ Dissociation constant (K_D)



Learn more about K_D

Storage buffer pH: 7.2 Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol, 0.05% BSA, PBS

Purity Protein A purified

Clonality Monoclonal
Clone number EPR4030(3)

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab133534 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	★★★★☆ (6)	1/10000 - 1/50000. Detects a band of approximately 37 kDa (predicted molecular weight: 37 kDa).
IHC-P	★★★★★ (3)	1/100 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
ICC/IF	**** <u>(5)</u>	1/1000. For unpurified use at 1/100 - 1/250.
IP		1/10 - 1/100.
Flow Cyt (Intra)		1/100 - 1/1000. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

Target

Tissue specificity

Function Plays an important role in homologous strand exchange, a key step in DNA repair through

homologous recombination. Binds to single and double-stranded DNA and exhibits DNA-

dependent ATPase activity. Catalyzes the recognition of homology and strand exchange between homologous DNA partners to form a joint molecule between a processed DNA break and the

repair template. Binds to single-stranded DNA in an ATP-dependent manner to form nucleoprotein filaments which are essential for the homology search and strand exchange

(PubMed:26681308). Part of a PALB2-scaffolded HR complex containing BRCA2 and RAD51C and which is thought to play a role in DNA repair by HR. Plays a role in regulating mitochondrial DNA copy number under conditions of oxidative stress in the presence of RAD51C and XRCC3.

Highly expressed in testis and thymus, followed by small intestine, placenta, colon, pancreas and

ovary. Weakly expressed in breast.

Involvement in disease Breast cancer

Mirror movements 2

Defects in RAD51 are found in a patient with microcephaly, mental retardation without bone

marrow failure and pediatric cancers.

Sequence similaritiesBelongs to the RecA family. RAD51 subfamily.

Contains 1 HhH domain.

Domain

The nuclear localization may reside in the C-terminus (between 259 and 339 AA).

Post-translational modifications

Ubiquitinated by the SCF(FBXO18) E3 ubiquitin ligase complex, regulating RAD51 subcellular location and preventing its association with DNA.

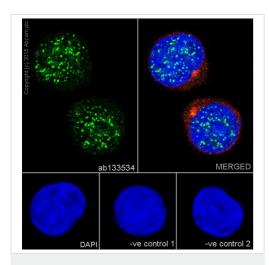
Phosphorylated. Phosphorylation of Thr-309 by CHEK1 may enhance association with chromatin at sites of DNA damage and promote DNA repair by homologous recombination.

Phosphorylation by ABL1 inhibits function.

Cellular localization

Nucleus. Cytoplasm. Cytoplasm, perinuclear region. Mitochondrion matrix. Cytoplasm, cytoskeleton, microtubule organizing center, centrosome. Colocalizes with RAD51AP1 and RPA2 to multiple nuclear foci upon induction of DNA damage. DNA damage induces an increase in nuclear levels. Together with FIGNL1, redistributed in discrete nuclear DNA damage-induced foci after ionizing radiation (IR) or camptothecin (CPT) treatment. Accumulated at sites of DNA damage in a SPIDR-dependent manner.

Images

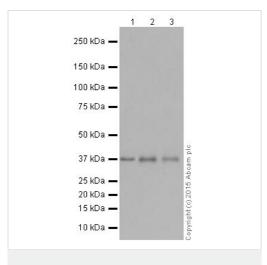


Immunocytochemistry/ Immunofluorescence - Anti-Rad51 antibody [EPR4030(3)] (ab133534)

Immunocytochemistry/Immunofluorescence analysis of Jurkat (human T cell leukemia cell line from peripheral blood) cells labelling Rad51 with purified ab133534 at 1/1000. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/1000) were also used.

Control 1: primary antibody (1/1000) and secondary antibody, **ab150120**, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: <u>ab7291</u> (1/1000) and secondary antibody, <u>ab150077</u>, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/1000).



Western blot - Anti-Rad51 antibody [EPR4030(3)] (ab133534)

All lanes : Anti-Rad51 antibody [EPR4030(3)] (ab133534) at 1/10000 dilution (purified)

Lane 1: C6 (rat glial tumor cell line) whole cell lysate

Lane 2: Mouse spleen tissue lysate

Lane 3: NIH/3T3 (mouse embryo fibroblast cell line) whole cell

lysate

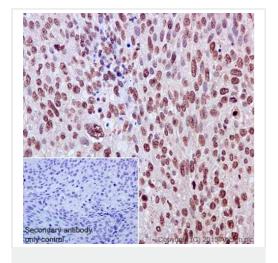
Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

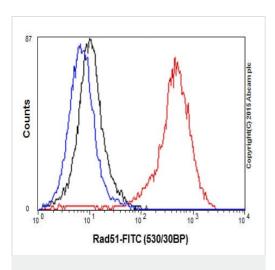
Predicted band size: 37 kDa **Observed band size:** 37 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



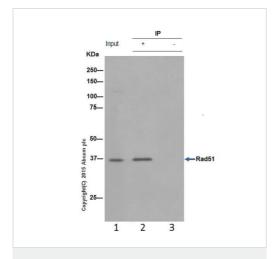
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Rad51 antibody
[EPR4030(3)] (ab133534)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervix carcinoma tissue labelling Rad51 with purified ab133534 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Flow Cytometry (Intracellular) - Anti-Rad51 antibody [EPR4030(3)] (ab133534)

Intracellular Flow Cytometry analysis of HeLa (human epithelial cell line from cervix adenocarcinoma) cells labelling Rad51 with purified ab133534 at 1/350 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit lgG (1/500) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal lgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Immunoprecipitation - Anti-Rad51 antibody [EPR4030(3)] (ab133534)

ab133534 (purified) at 1/100 immunoprecipitating Rad51 in HEK-293 (human epithelial cell line from embryonic kidney) whole cell lysate.

Lane 1 (input): HEK-293 whole cell lysate (10µg)

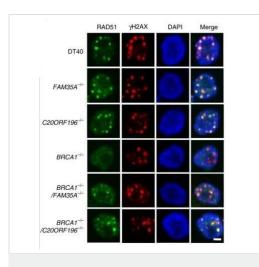
Lane 2 (+): ab133534 + HEK-293 whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab133534 in HEK-293 whole cell lysate.

For western blotting, a HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG was used as the secondary antibody (1/1500).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Immunocytochemistry/ Immunofluorescence - Anti-Rad51 antibody [EPR4030(3)] (ab133534)

Gao et al Nat Commun. 2018; 9: 3925. Published online 2018 Sep 25. doi: 10.1038/s41467-018-06407-7

Immunocytochemical analysis of DT40 cells (WT and the indicated KOs) labeling Rad51 using ab133534 at 1/200 dilution.

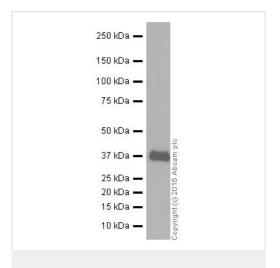
From Figure 5d of gao et al.

Gao et al Nat Commun. 2018; 9: 3925. Published online 2018

Sep 25. doi: 10.1038/s41467-018-06407-7

Reproduced under the Creative Commons

Licence http://creativecommons.org/licenses/by/4.0/.



Western blot - Anti-Rad51 antibody [EPR4030(3)] (ab133534)

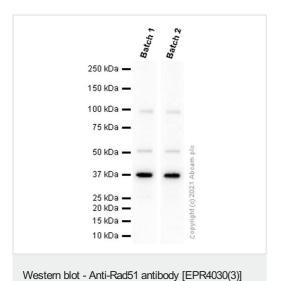
Anti-Rad51 antibody [EPR4030(3)] (ab133534) at 1/10000 dilution (purified) + K562 (human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate at 20 μ g

Secondary

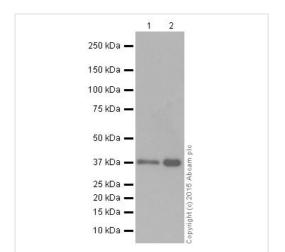
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 37 kDa **Observed band size:** 37 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Different batches of ab133534 were tested on HEK-293 (Human embryonic kidney epithelial cell) lysate at 2.1 μ g/ml. 15 μ g of lysate was loaded in each lane. Bands observed at 37 kDa.



(ab133534)

Western blot - Anti-Rad51 antibody [EPR4030(3)] (ab133534)

All lanes : Anti-Rad51 antibody [EPR4030(3)] (ab133534) at 1/20000 dilution (purified)

Lane 1 : HEK-293 (human epithelial cell line from embryonic kidney) whole cell lysate

Lane 2 : Jurkat (human T cell leukemia cell line from peripheral blood) whole cell lysate

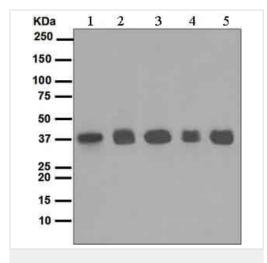
Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 37 kDa **Observed band size:** 37 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Rad51 antibody [EPR4030(3)] (ab133534)

All lanes : Anti-Rad51 antibody [EPR4030(3)] (ab133534) at 1/10000 dilution (unpurified)

Lane 1: C6 (rat glial tumor cell line) cell lysate

Lane 2: HEK-293T (human epithelial cell line from embryonic

kidney transformed with large T antigen) cell lysate

Lane 3 : Jurkat (human T cell leukemia cell line from peripheral blood) cell lysate

Lane 4 : HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate

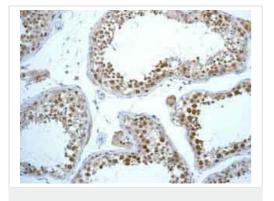
Lane 5 : K562 (human chronic myelogenous leukemia cell line from bone marrow) cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: HRP-conjugated goat anti-rabbit lgG at 1/2000 dilution

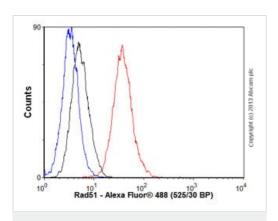
Predicted band size: 37 kDa **Observed band size:** 37 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Rad51 antibody
[EPR4030(3)] (ab133534)

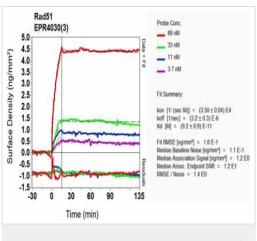
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human testis tissue labelling Rad51 with unpurified ab133534 at a dilution of 1/100.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-Rad51 antibody [EPR4030(3)] (ab133534)

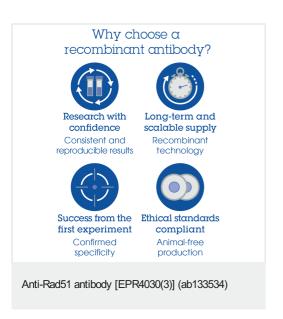
Overlay histogram showing HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained with unpurified ab133534 (red line). The cells were fixed with 80% methanol (5 minutes) and then permeabilized with 0.1% PBS-Tween for 20 minutes. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (unpurified ab133534, 1/1000 dilution) for 30 minutes at 22°C. The secondary antibody used was Alexa Fluorr 488 goat anti-rabbit lgG (H+L) (ab150077) at 1/2000 dilution for 30 minutes at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1 μ g/1x106 cells) used under the same conditions. Unlabeled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



OI-RD Scanning - Anti-Rad51 antibody [EPR4030(3)] (ab133534)

Equilibrium disassociation constant (K_D) Learn more about K_D

Click here to learn more about K_D



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- 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.

Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors