abcam

ab11055

Anti-RAD54 antibody [Rad54 4E3/1]

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	Preservative: 0.02% Sodium azide Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Full Length Protein corresponding to Human RAD54L. The exact immunogen used to generate this antibody is proprietary information. Database link Q92698
Clone number	Rad54 4E3/1
Purification technique	Affinity purification Protein A
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	1 µg for 10^6 Cells
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

WB

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information RAD54L

Function

Multifunctional ATPase that plays a role in homologous recombination (HR) which is a major pathway for repairing DNA double-strand breaks (DSBs), single-stranded DNA (ssDNA) gaps, and stalled or collapsed replication forks (PubMed:11459989, PubMed:12205100, PubMed:24798879, PubMed:27264870, PubMed:32457312, PubMed:9774452). Acts as a molecular motor during the homology search and guides RAD51 ssDNA along a donor dsDNA thereby changing the homology search from the diffusion-based mechanism to a motor-guided mechanism. Also plays an essential role in RAD51-mediated synaptic complex formation which consists of three strands encased in a protein filament formed once homology is recognized. Once DNA strand exchange occured, dissociates RAD51 from nucleoprotein filaments formed on dsDNA (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	RAD54 also known as DNA repair and recombination protein RAD54-like (Swiss-Prot Q9Y620) plays a mechanical role in DNA repair processes. It is part of the RAD52 epistasis group and shows DNA-dependent ATPase activity. RAD54 belongs to the DEAD-like helicase superfamily. It is approximately 82 kDa in mass and gets expressed in normal human tissues with higher levels in the testis. RAD54 has an ATPase activity modulating chromatin architecture making the DNA more accessible for repair enzymes.
Biological function summary	RAD54 contributes to homologous recombination an important mechanism for repairing double-strand breaks in DNA. It forms a complex with RAD51 facilitating RAD51-mediated homologous pairing and strand exchange. Through this interaction RAD54 promotes efficient DNA synapsis stabilizing the formation of joint molecules and protecting the DNA from further damage. RAD54 also unwinds DNA and removes RAD51 from dsDNA thereby resolving recombination intermediates.
Pathways	RAD54 functions in the homologous recombination repair pathway. This pathway involves several key proteins including BRCA1 and RAD51 which collaborate to repair double-strand DNA breaks and maintain genome stability. RAD54's work with these molecules ensures the accurate repair of damaged DNA. RAD54 also links to the Fanconi anemia pathway contributing to DNA damage response and crosslink repair.
Associated diseases and disorders	RAD54 associates with increased cancer susceptibility due to its role in DNA repair. Mutations or dysregulation of RAD54 can lead to genomic instability resulting in cancer types like breast cancer. RAD54 links to BRCA1 in this context highlighting its importance in preserving DNA integrity. Studies also show connections between RAD54 and developmental disorders resulting from impaired DNA repair mechanisms that affect cell growth and division.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

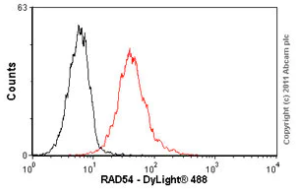
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

3 product images



Flow Cytometry - Anti-RAD54 antibody [Rad54 4E3/1] (ab11055)

RAD54 Flow Cytometry staining using mouse Anti-RAD54 antibody

Overlay histogram showing HeLA cells stained with ab11055 (red line). The cells were fixed with 100% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. 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 (ab11055, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was Mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

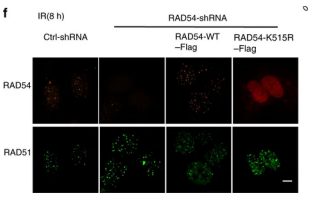


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Immunocytochemistry/ Immunofluorescence - Anti-RAD54 antibody [Rad54 4E3/1] (ab11055)

RAD54 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-RAD54 antibody

Immunocytochemistry-immunofluorescence using Anti-RAD54 antibody [Rad54 4E3/1], ab11055. Publication image from Zhang, Y. et al., 2020, Nat Commun, 32457312. Legend direct from paper.

RAD54 acetylation is important for BRD9 recognition and HR activity.a–c RAD54 is acetylated by GCN5/PCAF and deacetylated by HDAC 6/HDAC11 following induction of DNA damage. a 293T cells were transfected with control or RAD54-HA plasmid. Twenty-four hours after transfection, cells were exposed to the 10-Gy IR and harvested at the indicated time points. Immunoprecipitation with anti-HA beads was performed. Blots were probed with the indicated antibodies. b 293T cells were transfected with the indicated plasmids. Twenty-four hours after transfection, cells were

exposed to 10-Gy IR, and lysates were collected after 8 h. Immunoprecipitation with anti-HA beads was performed. Blots were probed with the indicated antibodies. c 293T cells were transfected with the indicated plasmids, treated as indicated, and subjected to immunoprecipitation as outlined in b. Blots were probed with the indicated antibodies. d, e RAD54 K515 acetylation is important for RAD51–RAD54 complex formation. d 293T cells were transfected with the indicated plasmids, treated as indicated, and subjected to immunoprecipitation as outlined in b. Blots were probed with the indicated antibodies. e 293T cells were transfected with the indicated plasmids, exposed to either no IR or 10-Gy IR as indicated, and subjected to immunoprecipitation as outlined in b. Blots were probed with the indicated antibodies. f–h RAD54 K515 acetylation is essential for HR activity. f, g U2OS cells were transfected with the indicated plasmids and exposed to 2-Gy IR. Cells were fixed after 8 h and stained for the indicated proteins. Representative immunofluorescence images of RAD51 (green) and RAD54 (red) are shown in f. Quantification of the indicated foci is shown in g. Representative data (mean ± SEM) are shown from n = 50 cells examined over three independent experiments. **p < 0.01,***p < 0.001 by two-sided unpaired t test. NS not significant. Scale bar, 10 µm. h Survival curves of U2OS cells expressing the indicated constructs and exposed to the indicated doses of PARPi or cisplatin. Representative data (mean ± SEM) are shown from n = 3 biologically independent samples. *p < 0.05, ***p < 0.001 by two-sided unpaired t test.

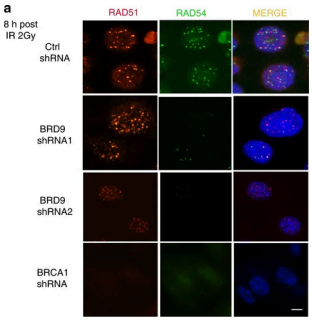


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Immunocytochemistry/ Immunofluorescence - Anti-RAD54 antibody [Rad54 4E3/1] (ab11055)

RAD54 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-RAD54 antibody

Immunocytochemistry-immunofluorescence using Anti-RAD54 antibody [Rad54 4E3/1], ab11055. Publication image from Zhang, Y. et al., 2020, Nat Commun, 32457312. Legend direct from paper.

BRD9 is required for interaction and co-localization of RAD51 and RAD54.a–d Representative immunofluorescence images of OVCAR8 cells demonstrating that BRD9 knockdown reduces RAD54 foci formation and RAD51/RAD54 co-localization. a–c OVCAR8 cells were infected with lentivirus-expressing control (Ctrl) or BRD9 shRNA and exposed to 2-Gy IR. Eight hours later, cells were stained with RAD51 (red) and RAD54 (green) antibodies. Representative immunofluorescence images are shown in a. RAD51 foci/cell (b), RAD54 foci/cell (c), and RAD51/RAD54 co-localized foci (d) after the indicated treatment were quantified. Representative data (mean ± SEM) are shown from three independent experiments. n = 50 cells examined over three independent experiments. ***p < 0.001 by two-sided unpaired t test. Scale bar, 10 µm. e, f DNA damage-induced RAD51–RAD54 interaction is dependent on BRD9. Endogenous BRD9 was knocked out in HEK293T cells using shRNA, and the indicated constructs were expressed. Cells were exposed to 10-Gy irradiation. Lysates were collected after 8 h, and immunoprecipitation with the indicated antibodies was performed. Blots were probed with the indicated antibodies. g RAD51 is necessary for BRD9 foci formation. OVCAR8 cells were infected with lentivirus-expressing RAD51 shRNA, exposed to 2-Gy IR, and fixed after 8 h. Cells were stained with BRD9 (green) and RAD51 (red) antibodies. Representative images of the indicated foci are shown. BRD9 foci/cell were quantified. Representative data (mean ± SEM) are shown from three independent experiments. n = 50 cells examined over three independent experiments. ***p < 0.001 by two-sided unpaired t test. Scale bar, 10 µm.

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