

Anti-Rad52 antibody, rabbit polyclonal, affinity-purified

70-015 50 μg

Shipping and Storage: Shipped at 4°C or -20°C and store at -20°C. Do not freeze.

Immunogen: Full-size recombinant human Rad52 protein, no tag-peptide attached...

Form; 1.0 mg/ml PBS- with 50% glycerol

Purity: Affinity-purified with recombinant Rad52 protein

Reactivity: human, mouse, rat, hamster

Applications

1) Western blotting (1/1,000~1/3,000)

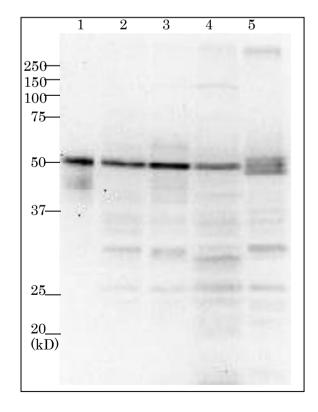
2) Immuno-precipitation (1/50-1/1,000))

3) Immunofluorescence staining (1/1,000)

4) ELISA (Assay dependent)

Background: Human Rad52 protein is a functional and structural homolog of yeast Rad52 protein, which plays a major role in genetic recombination and recombination repair with the RAD51 recombinase by mediating strand annealing between homologous DNA strands. Rad52 functionally and physically interacts with Rad51 in recombination processes. Rad52 foci formation is induced by DNA damage

Data Link: UniProtKB P43351 (RAD52_HUMAN)



Fig,1 Western Blot of Rad52 in mammalian cells

M: Marker proteins

- 1: Recombinant human Rad52 protein (0.2 ng)
- 2: HeLa cell extrat (17 ug)
- 3. MCF7 cell extract (27 ug)
- 4. NIH3T3 cell extract (20 ug)
- 5. CHO cell extract (21 ug)

.The samples were separated by electrophoresis on 10% SDS-PAGE and blotted onto **PVDF** membrane. The anti-Rad52 antibody was used at 1/1,000 dilution and as the second antibody, HRP-conjugated goat anti-rabbit IgG (ab97051) was used at 1/10,000 dilution Molecular mass of human Rad52 protein is 47 kDa



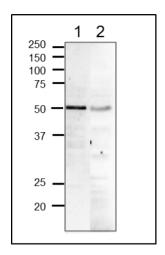


Fig.2 Weastern blot of Rad52 in Rat cells

- 1. Human MCF-7 cell lysate 16 $\,\mu$ g
- 2. Rat PC-12 cell 40 μ g

Proteins in the lysates were separated on SDS-PAGE (12.5%) and electro-blotted to PVDF membrane overnight. The blot was blocked with 5% skim milk. Anti-Rad52 antibody was used at 1/1,000 dilution and as the second antibody, HRP conjugated goat anti-rabbit IgG (ab97051) was used at 1/10,000 dilution.

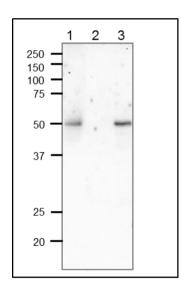


Fig.3 Immunoprecipitation of Rad52 from human cell lysate.

- 1.Add 10 µg of anti-Rad52 antibody and 50µg of Dynabeads proteinA
- 2. Incubate for 10 min at room temperature, wash and resuspend in $100~\mu l$ of the same buffer.
- 3. Add 100 µl (300 µg in PBS) lysate of human MCF7 cells
- 4.Incubate for and collect the immunocomplex with Dynabeeds magnetic apparatus
- 5. Elute the immunocomplex and analyze the presence of Rad52 in the supernatant (lane 2) and the eluate (lane 1) by western blotting with anti-Rad52 antibody.
- 6. As the second antibody, HRP-conjugated goat anti-rabbit IgG VeriBlot for IP (ab131366) was used at 1/10,000 dilution. Lane 3 is western blot of MCF' lysate (16 μ g)

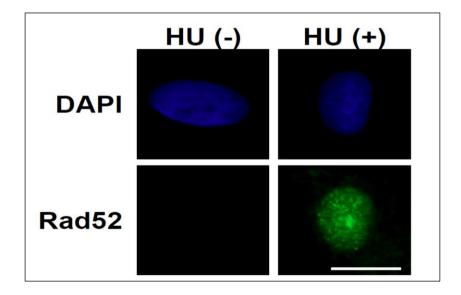




Fig.4 HU-induced focus formation of Rad52.

U2OS 40-2-6 cells were untreated or treated with 2 mM hydroxyurea (HU) for 24 h. The cells were pre-extracted with 0.1% TritonX-100 in PBS, fixed with 3.7% formaldehyde in PBS, and immunostained with anti-Rad52 antibody (green) at 1/50 dilution, followed by DAPI staining (blue). The second antibody was CF488A Goat anti-Rabbit IgG (Biotium) used at 1/1,000 dilution. TritonX-100 treatment extracts chromatin-free Rad52 from nuclei. Scale bars, 20 µm. The image is by courtesy of Prof .M. Fujita of Kyushu University.

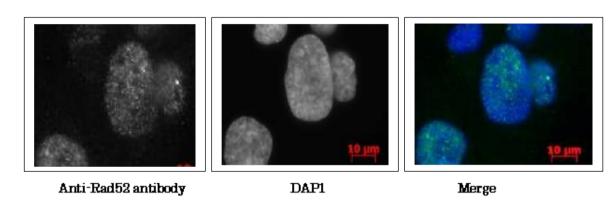


Fig.5. Detection of Ra52 protein in human fibroblast cell line, GM0637 by immunofluorescence staining

Cells were fixed with 4% paraformaldehyde in 1x PBS for 10 min, washed 3 times with PBS for 3 min, permealized by treatment with solution containing 1% SDS and 0.5% Triton for 10 min, washed 3 times with PBS for 3 min, incubated with anti-Rad52 antibody for 30 min at 37°C, washed 3 times with PBS for 3 min, incubated with secondary antibody for 30 min at 37°C, washed 3 times with PBS for 3 min, stained with DAPI for 1 min and mounted. Photographed with confocal microscope.

Anti-Rad52 antibody was used at 1/1,000 dilution. As the secondary antibody, goat anti-rabbit IgG conjugated with Alexa 488 was used at 1/2,000 dilution. The pictures were by courtesy of Prof. S. Tashiro and Mr. A. Fukuto at Hiroshima University.



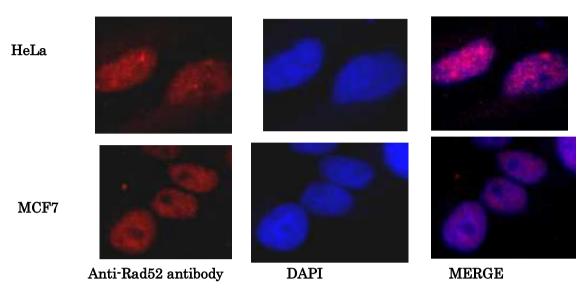


Fig.6 Detection of Rad52 protein in HeLa cells and MCF7 cells by immunofluorescence staining Fixed with 4% paraformaldehyde, and permeabilized with 0.25% TritonX-100. DNA was stained with DAPI. Anti-Rad52 antibody was used at 1/1,000 dilution and second antibody, goat anti-rabbit IgG Alex 488 at 1/1,000 dilution.

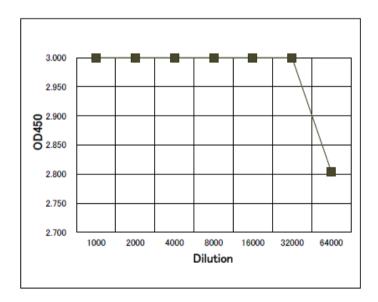


Fig 7 ELISA of human Rad52 protein with anti-Rad52 antiboy

ELISA analysis of human Rad52 protein, using the anti-Rad52 antibody. Plate was coated with 100 μl of recombinant human Rad52 protein at 1 μg/ml (BioAcademia 10-003) per well and 100 μl of the anti-Rad52 antibody at the indicated dilution was added to each well and incubated. After washing, goat anti-rabbit-IgG conjugated with HRP was added as a secondary antibody. Color was developed with TMB as substrate.



Reference: There has been no publication yet using this antibody.

Related Products

70-001 Anti-Rad51 antibody, rabbit-serum (50 ul)

70-002 Anti-Rad51 antibody, rabbit-serum (250 ul)

70-005 Anti-Rad51 antibody, Chicken IgY

70-012 Anti-Rad51 antibody, rabbit polyclonal, affinity-purified, ChIP grade

BioAcademia,Inc. Tel. 81-6-6877-2335 Fax. 81-6-6877-2336 <u>info@bioacademia.co.jp</u> <u>http://www.bioacademia.co.jp/en/</u>