

Anti-Rad51 antibody (rabbit polyclonal, affinity-pure) ChIp grade

70-012 100 μg

Storage: Shipped at 4° C and store at -20° C (Do not store below -20° C to avoid freezing)

Reactivity: human, rodents, chicken, frog (Xenopus)

Immunogen: Full-size recombinant human Rad51 protein (BioAcademia <u>10-001</u>) **Applications**

- 1) Western blotting $(1/1,000 \sim 1/10,000 \text{ dilution})$. Fig. 1
- 2) Immunoprecipitation(1/1,00-1/1,000). Fig. 2
- 3) Chromatin Immunoprecipitation (ChIp) (assay dependent)
- 4) Immuno-fluorescent staining (1/1,000~1/10,000). Fig. 3
- 5) Immunohistochemistry (1/100-1/1,000). Fig.4
- 6) Dot blotting (1/1,000~1/5,000 dilution)
- 7) ELISA (indirect, 1/2,000~1/5,000 dilution) Not tested for other applications

Purity: Affinity-purified with full-size recombinant human Rad51 protein **Form:**1 mg/ml in PBS with 50% glycerol, filter-sterilized. Azide-and carrier-free

Backgroud: Human Rad51 protein is a functional and structural homolog of *E.coli* RecA protein, which plays a major role in genetic recombination and recombination repair by mediating strand exchange reaction between homologous DNA strands (Ref.1). Rad51 functionally and physically interacts with its paralogs Dmc1, Rad51B, Rad51D, Xrcc2 and Xrcc3, and also with Rad52 in recombination processes. It also interacts with oncogene products and tumor suppressors such as BRACA1, BRACA2, and p53 for the maintenance of genome stability.

References: Please let us know any publications using this antibody.

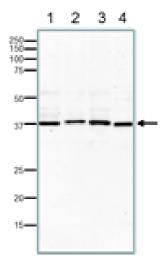


Fig.1 Western blotting of endogenous Rad51 proteins in crude extracts of varius animal cells.

15% SDS-PAGE was used. Blotting was done at 15 v overnight with wet system. The antibody was used at 1/1,000 dilution.

- 1. MCF7 (human) cells. 40 $\,\mu$ g
- 2. NIH3T3 (mouse) cells. 40 μ g
- 3. CHO (hamster) cells. 40 $\,\mu$ g
- 4, Xenopus laevis (frog) egg. 40 μ g



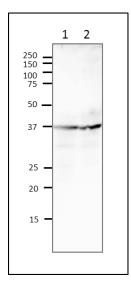


Fig.2. Western blot of endogenous Rad52 protein in rat cell lysate.

- 1. Human MCF7 cell lysate (80 µg)
- 2. Rat PC12 cell lysate (80 µg)

Proteins in the lysates were separated on SDS-PAGE (12.5 % gel), and blotted to PVDF membrane at 15 V overnight. The membrane was blocked with 3% skim milk. The anti-Rad51 antibody was used at 1/1,000 dilution and as the second antibody, goat anti-rabbit IgG (ab97051) was used at 1/10,000 dilution.

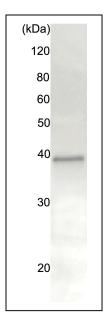


Fig.3. Immunoprecipitation of Rad51 protein from crude extract of HeLa cells by the anti-Rad51 antibody.

Anti-Rad51 antibody (20 ug) was incubated with 20 $\,\mu$ g of HeLa cell extract, and precipitated with 20 $\,\mu$ g of proteinA-beads. The sample was dissociated from the precipitate by heating in SDS-sample buffer and analyzed by western blotting with anti-Rad51 antiserum (chicken, ab63802) at 1/1,000 dilution. As second antibody, anti-chicken IgG antibody (rabbit) was used at 1/10,000 dilution.



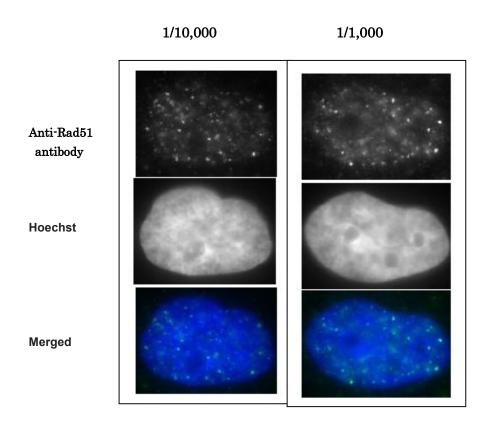


Fig.4. Detection of Rad51 foci formation after X-ray irradiation in human fibroblast cell line, GM0637.

Cells were irradiated by X-rays at 2 Gy,, grown for 1 hr, fixed with 4% paraformaldehyde in 1x PBS for 10 min, washed 3 times with PBS for 3 min, permealized by treatment with 0.5% Triton for 5 min, washed 3 times with PBS for 3 min, incubated with anti-Rad51 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 Hoechst for 1 min and mounted.

Anti-Rad51 antibody was used at 1/10,000 dilution (left panels) and 1/1,000 dilution (right panels). As the secondary antibody, anti-rabbit Alexa 488 was used at 1/10,000 dilution. The pictures were by courtesy of Prof. S. Tashiro and Dr. K. Kono at Hiroshima University.



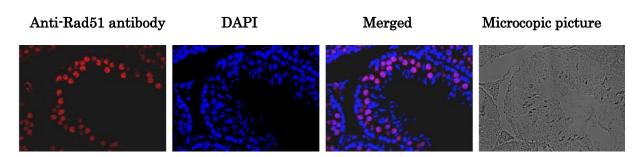


Fig.5. Immunohistological staining of Rad51 protein in mouse testis using anti-Rad51 antibody. A section of formalin fixed and paraffin embedded mouse testis was treated with the anti-Rad51 antibody at 1/100 dilution after deparaffization and antigen retrieval. The 2nd antibody, anti-rabbit IgG conjugated with Alexa Fluor 647 (Abcam) was used at 1/1,000 dilution. DNA was stained with DAPI and the merged image was shown (Merged). The plain black and white microsopic picture of the same region was shown on the right.

Related Products:

10-001 Rad51 protein (human)

10-003 Rad52 protein (human)

70-001 anti-Rad51 (hu,,ms,rat, etc) antibody, rabbit serum

70-003 anti-Rad51 (hu,ms,rat,etc) antibody, chicken serum

70-005 anti-Rad51 (hu,ms,rat,etc)) antibody, IgY

70-015 anti-Rad52 (hu,ms,rat) antibody, rabbit polyclonal, affinity purified