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Catalog No. 70-001EX

# Anti-Rad51 (human) antibody (rabbit serum)

#### **BACKGROUND**

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 oncogenes and tumor suppressors such as BRACA1, BRACA2, and P53 for the maintenance of genome stability (Ref.1).

The product was prepared by immunizing rabbit with full-size recombinant Rad51 protein expressed in *E.coli* and purified. Using this anti-serum, Rad51 protein (37kD) in the crude extract of HeLa cells was detected by Western blotting (Fig.1) and Rad51 foci formation induced by stalled replication and DNA damage was detected by indirect immunofluorescence (Fig.2 & Ref.2). GFP-tagging of Rad51 protein at either N- or C- terminus inactivates the function of Rad51, and therefore, it cannot be used for the study of foci formation instead of the antibody.

### **Applications**

- 1) Western blotting (x 2,000~x 10,000 dilution). High specificity
- 2) Immunoprecipitation
- 3) Detection of foci formation by indirect immunofluorescence method

#### **Specification**

Reactivity: Reacts with human, mouse and chicken Rad51

Form: 0.09% sodium azide added

Size: 50 ul

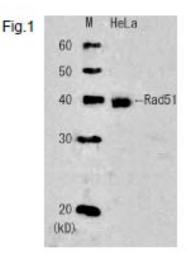
Storage: Shipped at 4°C or -20°C and stored at -20°C.

Data Link UniProtKB/Swiss-Prot Q06609 (RAD51\_HUMAN)

References: This antibody has been used in Ref. 2 and 3.

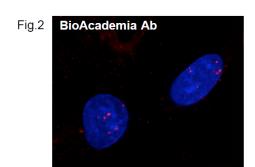
- 1. Tashiro S *et al*, Rad51 accumulation at sites of DNA damage and in postreplicative chromatin. *J Cell Biol* **150**: 283-291 (2000) PMID: 10908572
- Nakano T et al, Homologous Recombination but Not Nucleotide Excision Repair Plays a Pivotal Role in Tolerance of DNA-Protein Cross-links in Mammalian Cells. J. Biol. Chem. 284:27065-27076.
- 3. Vaz F et al, Mutation of the Rad51C gene in a Fanconi anemia-like disorder. Nature Genetcs 42:406-409 (2010)

PMID: 20400963





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# Fig.2 Detection of Rad51 foc: formation induced by DNA damage.

Normal human diploid cells were irradiated by X-ray (0.5 Gly) and after 6 hr incubation, the cells were fixed and immunostained by using anti-Rad51 serum (x 100 dilution) as the primary antibody and Alexa 594 labeled anti-rabbit antibody as the secondary antibody.

(The picture was kindly provided by Prof. K. Suzuki, Medical School of Nagasaki Univ.)

#### **Related Products:**

10-001EX Rad51 protein (human)

10-003EX Rad52 protein (human)

70-003EX anti-Rad51 (human) antibody, chicken antiserum

70-005EX anti-Rad51 (human) antibody, IgY

70-007EX anti-Rad51 (human) antibody, Sepharose-conjugated IgY

70-009EX anti-Rad51 (human), antigen affinity-purified IgY

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Inspiration for Life Science

TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN



# Anti-Rad51 (human) antibody (rabbit serum)

Detailed protocol for the use of this antibody is provided by Prof. K Suzuki as follows.

Protocol for the use of anti-Rad51 (human) antibody, rabbit serum, for immuno-staining to observe foci-formation on chromosome after incubation of DNA-damaged cells as follows.

- 1. Incubate cells on glass slips for at least 24 hours.
- 2. Wash cells with PBS once.
- 3. Fix cells with cold methanol for 10 min on ice.
- 4. Wash cells with PBS.
- 5. Incubate cells with 0.5% Triton X-100 for 5 min on ice.
- 6. Wash cells with PBS extensively.
- 7. Incubate cells with anti-Rad51 antibody diluted (1:100) in TBS-DT (TBS: 20 mM Tris-HCl, pH7.6, 137 mM NaCl, containing 0.1% Tween-20 and 5% Skim milk).
- 8. Wash cells with PBS.
- 9. Incubate cells with Alexa594-labeled anti-rabbit antibody diluted (1:1000) in TBS-DT.

In addtion to the above;

- 1. Different fixation methods have to be employed depending on types of cells.
- 2.Different blocking reagents have to be tried in case of high background.
- 3. In the absence of DNA-damage, Rad51 is localized diffusely in whole nucleus but Rad51 accumulates as foci on chromosome with double strand DNA breaks or stalled replication forks.

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