Affinity-purified Sheep Anti-human/rat Rad17 Antibody

ORDERING INFORMATION

Catalog Number: AF1926

Lot Number: JUK01

Size: 100 µg

Formulation: 0.2 µm filtered solution in PBS with 5% trehalose

Storage: -20° C

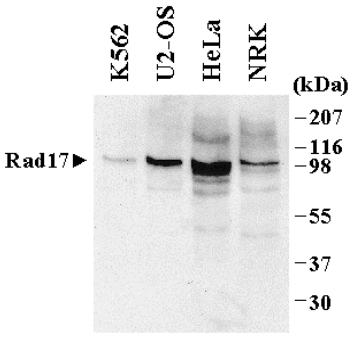
Reconstitution: sterile PBS and 0.02% NaN_3

Specificity: human/rat Rad17

Immunogen: *E. coli*-derived recombinant human Rad17 (amino acids 1 - 240)

Ig Type: affinity-purified sheep IgG

Applications: Western blot
Immunoprecipitation



Extracts from exponentially growing human cell lines (K562, U2-OS, or HeLa), or normal rat kidney (NRK) cells were prepared, resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunblotted with 1.0 µg/mL sheep anti-Rad17 antibody.

Background

The DNA damage response activates cell cycle checkpoints to allow time for DNA repair and ensure the fidelity of each cell cycle. The Rad genes were first identified in yeast as genes required for the DNA damage response. Human Rad17 bears homology to the replication factor C (RFC) proteins and interacts with the Rad9/Rad1/Hus1 complex in cells exposed to multiple types of genotoxic stress, including ionizing radiation (IR) and ultraviolet light (UV).

Preparation

Sheep antibodies were raised against purified, *E. coli*-derived, recombinant human Rad17 (rhRad17). Polyclonal antibody was affinity-purified on a column derivatized with rhRad17, and further purified by isolating the IgG fraction.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) containing 5% trehalose.

Reconstitution

Reconstitute the antibody with 100 µL of sterile PBS containing 0.02% NaN_3.

Storage

The reconstituted antibody should be aliquoted and stored at -20° C **in a manual defrost freezer** until use**. Avoid repeated freeze/thaw cycles.**

Specificity

The antibody detects human and rat Rad17.

Applications

Western blot - An antibody concentration of 1.0 µg/mL is recommended.

Immunoprecipitation - Use 3 µg antibody per 500 µg cell extract.

Protocols for Immunoprecipitation and Immunoblotting:

Immunoprecipitation

Wash Buffer	Cell Lysis Buffer
50 mM Tris, pH 7.4	Wash Buffer containing:
150 mM NaCl	3 µg/mL aprotinin
1 % NP-40	2 µg/mL leupeptin
1 mM DTT	2 µg/mL pepstatin A

Cell lysates for immunoprecipitation: Wash cells twice with cold PBS and extract cell protein by solubilization of 1 x 10^6 - 5 x 10^6 cells in 1 ml cold Cell Lysis Buffer. Solubilize cells for 15 minutes on ice, followed by centrifugation at 6000 x g for 5 minutes to clear insoluble material. Measure protein concentration and bring volume up to 1 mL per sample with Cell Lysis Buffer.

Immunoprecipitation: Add 3 µg sheep anti-Rad17 per 500 µg extract and incubate 1 hour on ice with occasional inversion. Add Protein G agarose (20 µL of a 50% suspension) to each sample and rotate 1 hour at 4° C. Pellet the Protein G-absorbed complexes and wash twice with Wash Buffer. Suspend the washed pellet in 25 - 50 µL 2X SDS gel sample buffer (see above) and incubate 5 minutes in boiling water bath. Pellet Protein G agarose and resolve the supernatant by SDS-PAGE.

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Western blotting

Blotting Buffer	Blocking Solution
25 mM Tris, pH 7.5	5% nonfat dry milk
0.15 M NaCl	in blotting buffer
0.05% Tween 20	pH to 7.5

1. Transfer the electrophoresed proteins onto a PVDF membrane and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane for 2 hours at room temperature or overnight at 2 - 8° C in Blocking Solution containing 1 μg/mL sheep anti-Rad17.
3. Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Blocking Solution containing a 1:7,500 dilution of HRP-conjugated rabbit anti-sheep Ig (Zymed).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with ECL Reagent.

Cell lysates for western blotting: To prepare total cell lysates, solubilize cells in 2X SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) and sonicate with a probe sonicator using 3-4 bursts of 5-10 seconds each. Heat extracts in a boiling water bath for 5 minutes and load onto polyacrylamide gels. Samples may be diluted with 1X SDS sample buffer to the desired concentration.

Optimal dilutions should be determined by the individual laboratory.