

Affinity-purified Goat Anti-human Rad50 Antibody

ORDERING INFORMATION

Catalog Number: AF4996

Lot Number: CBAM01

Size: 100 µg

Storage: -20° C

Specificity: human Rad50

Immunogen: *E. coli*-derived recombinant human Rad50 (rhRad50; aa 518 - 881)

Ig Type: affinity-purified goat IgG

Application: Western blot

Background

Rad50 is a 150 kDa member of the Rad50 subfamily, SMC (structural maintenance of chromosomes) family of DNA-associated genes. It is ubiquitously expressed, and associates with MRE11 and NBS1 to form an MRN complex. This complex stabilizes ATM kinase, thus contributing to DNA repair, and also participates in the suppression of DNA rereplication in dividing cells. Human Rad50 is 1312 amino acids (aa) in length. It has an apparent ATP binding site (aa 36 - 43) plus a coiled-coil region (aa 228 - 598) followed by a "zinc-hook" domain (aa 635 - 734) that mediates homodimerization. There are multiple splice variants. An alternate start site exists at Met140, there is a single Lys substitution for aa 723 - 1312, and three Lys substitute for aa 555 - 1312. Over aa 518 - 881, human Rad50 is 96% aa identical to mouse Rad50.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived, recombinant human Rad50 (rhRad50; aa 518 - 881; Accession # Q92878). Polyclonal antibody was affinity-purified on a column derivatized with rhRad50 and further purified by isolating the IgG fraction.

Formulation

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

Reconstitution

Reconstitute the antibody with 100 µL of sterile PBS containing 0.02% NaN₃.

Storage

Lyophilized samples are stable for 12 months from date of receipt when stored at -20° C to -70° C. The reconstituted antibody should be aliquoted and stored at -20° C in a manual defrost freezer for 12 months without detectable loss of activity. **Avoid repeated freeze/thaw cycles.**

Specificity

The antibody detects endogenous human Rad50 in Western blots.

Application

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

Protocols for Immunoblotting

Blotting Buffer

25 mM Tris, pH 7.5

0.15 M NaCl

0.05% Tween® 20

Blocking Solution

5% nonfat dry milk

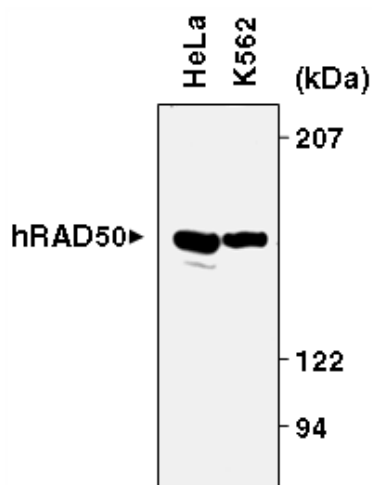
in Blotting Buffer

Adjust 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 overnight at 2° - 8°C in Blocking Solution containing 1.0 µg/mL goat anti-hRad50 antibody.
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 1:1,000 dilution of HRP-conjugated donkey anti-goat Ig (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

Cell lysates for Western blottings - To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF, and bromophenyl blue) at 2 x 10⁶ - 1 x 10⁷ cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each.

Optimal dilutions should be determined by each laboratory for each application.



Extracts from 1.5 x 10⁵ exponentially growing HeLa and K562 cells were prepared, resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunoblotted with 1.0 µg/mL goat anti-hRad50 antibody.