Affinity-purified Rabbit Anti-Phospho-SMC1 (S966) Antibody

ORDERING INFORMATION

Catalog Number: AF2677

Lot Number: VAI01

Size: 50 μg

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

Storage: -20° C

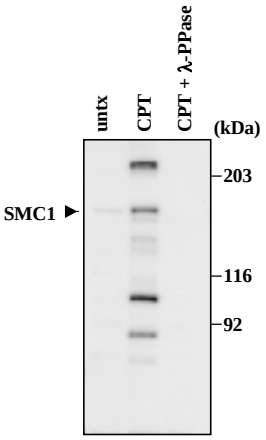
Reconstitution: sterile PBS and 0.02% NaN_3

Specificity: human phospho-SMC1 (S966)

Immunogen: phosphopeptide containing human SMC1 phosphorylated at the S966 site

Ig Type: rabbit IgG

Application: Western blot



Exponentially-growing U2-OS cells were left untreated (untx) or treated with 1 μM camptothecin (CPT), and whole cell extracts were prepared 4 hours later. The indicated sample was treated with 600 U λ-phosphatase (λ-PPase, NEB). Equal amounts of cell extracts were resolved by SDS-PAGE, transferred to PVDF membrane, and immunoblotted with 0.5 μg/mL anti-phospho-SMC1 (S966). This antibody detects additional proteins in a phospho-specific manner (CPT but not CPT + λ-PPase), which likely indicates reactivity with other phospho-S/Q residues.

Background

The ATM protein kinase phosphorylates the structural maintenance of chromosomes (SMC1) protein in cells exposed to genotoxins. Overexpression of a non-phosphorylatable mutant of SMC1 subjects a cell to a defective S phase checkpoint and decreased cell survival in response to genotoxic stress. In cells expressing this mutant SMC1, BRCA1 and Nbs1 are still phosphorylated, a fact that suggests SMC1 phosphorylation is downstream of these other phosphorylation events.

Preparation

Rabbit antibodies were raised against a synthetic phosphopeptide containing the phosphorylated serine at position 966 of human SMC1. Polyclonal antibody was affinity-purified on a column derivatized with the phosphopeptide, dialyzed, and concentrated.

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% 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 SMC1 phosphorylated at S966. The antibody does not recognize SMC1 unphosphorylated at S966.

Application

Western blot - An antibody concentration of 0.5 μg/ml is recommended.

Protocols for Immunoblotting:

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 an Immobilon membrane (Millipore) 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 0.5 μg/mL rabbit anti-phospho-SMC1 (S966).
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 1:2,000 dilution of HRP-conjugated goat anti-rabbit Ig (R&D Systems, Catalog # HAF008).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with Western Glo Chemiluminescent detection reagents (R&D Systems, Catalog # AR004) or equivalent.

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.

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R&D Systems, Inc.
1-800-343-7475

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