Affinity-purified Goat Anti-human Chk2 Antibody

ORDERING INFORMATION

Catalog Number: AF1358

Lot Number: HXF01

Size: 100 μg (for 100 mL blotting solution)

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

Storage: -20° C

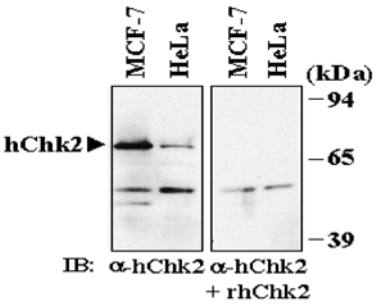
Reconstitution: sterile PBS and 0.02% NaN_3

Specificity: human Chk2

Immunogen: *E. coli*-derived recombinant human Chk2, amino acids 208 - 543

Ig Type: affinity-purified goat IgG

Application: Western blot



Extracts from exponentially growing MCF-7 or HeLa cells were prepared, resolved by SDS-PAGE, and transferred to an Immobilon membrane. The membrane was immunblotted with 1.0 μg/mL goat anti-hChk2 antibody (*left panel*) or 1.0 μg/mL goat anti-hChk2 antibody preincubated with recombinant human Chk2 (rhChk2) (*right panel*). Migration of the hChk2 protein is consistent with a molecular weight of ~ 65 kDa, and preincubation with rhChk2 depletes the anti-hChk2 immunoreactivity.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived, recombinant human Checkpoint kinase 2 (rhChk2). Polyclonal antibody was affinity-purified on a column derivatized with rhChk2 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% 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 Chk2.

Application

Western blot - An antibody concentration of 1.0 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 Immobilon membranes (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane for 4 hours at room temperature or overnight at 2 - 8° C in Blocking Solution containing 1.0 μg/mL goat anti-human Chk2.
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 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 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.

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