Affinity-purified Goat Anti-human/mouse/rat p53-Agarose

ORDERING INFORMATION

Catalog Number: GAF1355

Size: 250 µL beads in 500 µL suspension

Formulation: PBS with preservative

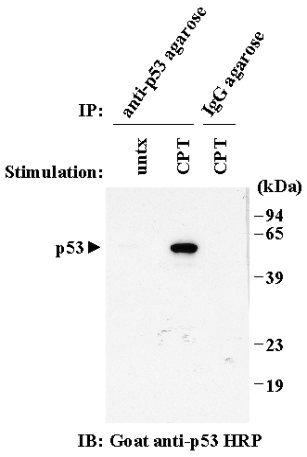
Storage: 2° - 8° C

Specificity: human/mouse/rat p53

Immunogen: *E. coli*-derived recombinant human p53

Ig Type: affinity-purified goat IgG

Application: Immunoprecipitation



MCF-7 cells were left untreated (untx) or incubated in the presence of 1 µM camptothecin (CPT) for 6 hours. Cell extracts were prepared in Cell Lysis Buffer, and incubated 1 hour with (*lanes 1-2*) goat anti-human/mouse/rat p53-Agarose or with (*lane 3*) normal goat IgG-Agarose. Absorbed extracts were washed, eluted, and resolved by SDS-PAGE. Proteins transferred to a PVDF membrane were immunoblotted (IB) with goat anti-human/mouse/rat p53-HRP (R&D Systems, Catalog # HAF1355).

Preparation

Goat antibodies were raised against purified, *E. coli*-derived, recombinant human p53 (rhp53), affinity-purified on a column derivatized with rhp53, and further purified by isolating the IgG fraction. The affinity purified antibody was conjugated to agarose.

Formulation

Phosphate-buffered saline (PBS) with preservative.

Storage

Upon receipt, centrifuge tube to pellet agarose that may have collected in the cap during shipment. The agarose should be stored at 2° - 8° C for up to twelve months without detectable loss of activity. **Do not freeze.**

Specificity

The antibody detects human, mouse, and rat p53.

Application

Immunoprecipitation - Use 10 µL beads (20 µL agarose slurry) per 500 µg total cell extract.

Buffers:

Wash Buffer	Cell Lysis Buffer	2X SDS Buffer
50 mM Tris, pH 7.4	Wash Buffer containing:	0.25 M Tris, pH 6.8
0.15 M NaCl	2 µg/mL aprotinin	6% SDS
1% NP-40	5 µg/mL leupeptin	10% glycerol
20 mM β-glycerophosphate	2 µg/mL pepstatin A	20 mM dithiothreitol
10 mM NaF		Bromophenyl Blue
1 mM DTT		

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 6,000 x g for 5 minutes to clear insoluble material. Measure protein concentration and bring 500 µg cell extract up to 1 mL per sample with Cell Lysis Buffer.

Protocols for Immunoprecipitation:

1. Add 20 µL goat anti-human/mouse/rat p53-Agarose slurry per 500 µg extract and rotate 1 hour at 2° - 8° C.
2. Pellet the Agarose-absorbed complexes by centrifugation at 4,000 x g for 30 seconds. Wash Agarose pellets twice with Wash Buffer.
3. Suspend the washed pellets in 25 - 50 µL 2X SDS Buffer and incubate 5 minutes in a boiling water bath.
4. Pellet Agarose and resolve the supernate by SDS-PAGE.

Visualization: Proteins from SDS-PAGE gels may be transferred to a PVDF membrane and visualized by Western blotting with goat anti-human/mouse/rat p53-HRP (R&D Systems, Catalog # HAF1355).

Optimal volumes should be determined by the individual laboratory.

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