

ORDERING INFORMATION

Catalog Number: AF3306

Lot Number: VUM01

Size: 50 µg

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

Storage: -20° C

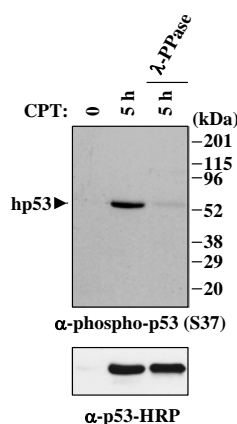
Reconstitution: sterile PBS and 0.02% NaN₃

Specificity: human phospho-p53 (S37)

Immunogen: phosphopeptide containing human p53 S37 site

Ig Type: rabbit IgG

Application: Western blot



MCF-7 cells were left untreated or treated with 1 µM camptothecin (CPT). Five hours after exposure, cellular extracts were prepared in 0.5% Triton X-100 lysis buffer, and incubated 2 hours with anti-p53 agarose (R&D Systems, Catalog # GAF1355). Agarose beads were washed, eluted, and resolved by SDS-PAGE. Proteins transferred to a PVDF membrane were immunoblotted with 0.4 µg/mL anti-phospho-p53 (S37). The membrane was stripped and reprobed with anti-p53 HRP (R&D Systems, Catalog # HAF1355) (*lower panel*).

Background

The p53 tumor suppressor protein acts to enforce cell cycle checkpoints or signal apoptosis in cells that have incurred genotoxic damage. The ATR and DNA-PK_{cs} kinases phosphorylate p53 at serine 37 in response to UV light and other genotoxic stressors. This phosphorylation event results in increased p53 transcriptional activation.

Preparation

Rabbit antibodies were raised against a synthetic phosphopeptide containing the phosphorylated serine at position 37 of human p53. 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) containing 5% trehalose.

Reconstitution

Reconstitute the antibody with 100 µl of sterile PBS containing 0.02% NaN₃ for a final concentration of 0.5 µg/µL.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

The antibody detects human p53 phosphorylated at S37. The antibody does not recognize p53 unphosphorylated at S37.

Application

Western blot - An antibody concentration of 0.4 µg/mL is recommended.

Protocols for Immunoblotting:

Western blotting

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
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 4 hours at room temperature or overnight at 2 - 8° C in Blocking Solution containing 0.4 µg/mL rabbit anti-phospho-p53 (S37).
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 goat anti-rabbit IgG (R&D Systems, Catalog # HAF008).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with WesternGlo™ Chemiluminescent detection reagents (R&D Systems, Catalog # AR004) or equivalent.

Optimal dilutions should be determined by the individual laboratory.