Anti-p53 acetyl-K382 antibody, monoclonal (2B7E4)

71-133　50　μg

p53 mutants are found in more than half of human cancers and are considered as the most important human cancer related gene. p53 is detected at 53kD position by electrophoresis and is composed of 393 amino acids. In the unstressed normal cells the p53 level is low and it is inactive. However, with stress, especially with DNA damage, it is activated to promote arrest of cell cycle and repair of DNA damage, or induction of apoptosis. The functions and stability of p53 are regulated by the phosphorylation of serine and threonine, and the acetylation of lysine at various sites in the molecule.

Acetylation of lysine 382 (acetyl-K382) of p53 occurs after DNA damage and is catalyzed by the p300/CBP acetyltransferase, which stabilize p53 proten (ref 1).

Applications

1) Wester blotting (~1 ug/ml))　2) ELISA

3) Immuno-precipitation and indirect immuno-staining have not been tested.

Specification

Antigen: synthetic peptide containing acetyl-Lys382 of human p53.

specificity: Reacts with human p53 acetylated at Lys382. Other species not tested

Isotype: mouse IgG1 (κ)

Form: Purified IgG 1mg/ml in PBS (pH 7.4), 50% glycerol, sterilized by filtration

Storage: -20 ℃ (long period, -70℃)

References:

1. Bode AM & Dong Z.　Post-translational modification of p53 in tumorigenesis. Nature Rev. Cancer 4:793 (2004)

Fig. Identification of p53 protein, whose Lys382 is acetylated, by Western blotting with 2B7E4 antibody.

MCF7 cells in culture were treated with actinomycin D at 5 nm for the indicated periods and the cell extracts were analyzed by western blotting with anti-p53 acetyl-K382 antibody (2B7E4) and omnipotent anti-p53 antibody (DO-1). Acetylation of p53 at K382 was induced by the DNA damaging treatment.

Fig.1

Related Product

71-131 anti-p53 Ac-K120 antibody, monoclonal (10E5)

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PROTOCOL: WESTERN BLOTTING

1. Grow the appropriate human cells such as MCF7 (breast cancer cell line) or Molt4 (leukemia cell line) to log phase and treat the celll with DNA damaging reagents such as actinomycin D or UV light. The cells are incubated for 3~9 h to induce acetylation of p53 in the presence of trichostatin (0.5 uM), an inhibitor of deacetylase. The untreated cells are used as control.
2. The cells are harvested by centrifugation and lysed by adding and incubating with lysis buffer (50 mM Tris-Hcl pH 7.5, 150 mM NaCl, 0.5% NP-40, 50 mM NaF, 1 mM Na_3VO_4, 1 mM DTT, 0.1 mg/ml E64, 0.1 mg/ml leupeptin, 0.1 mg/ml SBTI) on ice for 30 min. The supernatant is obtained by centrifugation in microcentrifuge at 10,000 rpm for 10 min.
3. The samples are added with SDS-sample buffer and separated by 10% SDS PAGE followed by transfer of proteins to Immobilon P-membrane (Millipore).
4. Immunoblotting is carried out using monoclonal antibody specific for p53 acetyl-K382 (clone 2B7E4) at 0.5~1.0 µg/ml followed by horseradish peroxidase-conjugated secondary antibody to mouse IgG and detection with enhanced chemiluminescence (ECL-plus, GE Healthcare Biosciences).

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