

Anti-p53 acetyl-K120 antibody, mouse monoclonal (10E5)

71-131 100 μg

Shipping and Storage: Shipped at 4° C or -20° C, and store at -20° C.

Immunogen: synthetic peptide containing acetyl-Lys315 of human p53

Form: purified monoclonal antibody (IgG) 1mg/ml in PBS- with 50% glycerol

Isotype: mouse $IgG1 \kappa$

Applications

- 1. Western blotting (~1 ug/ml) (Fig.1)
- 2. Immuno-precipitation (Fig.2)
- 3. Immunofluorescence staining (Fig.3)
- 4. Flow-Cytometry (1/100)
- 5. ELISA

Background: *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 phosphorylation of serine and threonine, and acetylation of lysine at various sites in the molecule.

Acetylation of lysine 120 (acetyl-K120) of p53 occurs rapidly after DNA damage and is catalyzed by the MYST family acetyltransferases hMOF and TIP60, and activates transcription of proapoptotic genes, BAX and PUMA.

Data Link UniProtKB/Swiss-Prot P04637 (P53 HUMAN)

Related Products: many antibodies specific to phosphorylated and acetylated oncogene products

#<u>71-113</u> anti-p53 (p-S20) #<u>71-115</u> anti-p53 (p-S46) #<u>71-117</u> anti-p53 (p-S315) #71-133 anti-p53 (Ac-K382)



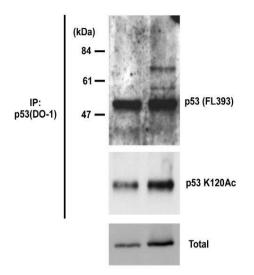


Fig. 1 Identification of p53 protein, whose Lys 120 is acetylated, by Western blotting with 10E5 monoclonal antibody.

Samples are crude lysates of HCT116 cells: Left lanes are control. Right lanes are cells treated with siRNA to knockdown the expression of a Tip60 interacting protein UHRF1, which results in increase in acetylation of p53 at Lys120. Total p53 was immuno-precipitated with omnipotent anti-p53 monoclonal antibody (DO-1) from the crude extracts and analyzed by Western blotting with anti-p53 antibody (FL393) (upper panel) or anti-p53 acetyl-K120 monoclonal antibody (10E5) (middle panel). The lower panel shows total p53.

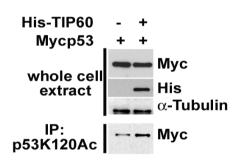


Fig.2 Immunoprecipitation of p53 acetylated at K120 by 10E5 monoclonal antibody.

Crude cell extracts were prepared from H129 cells (p53 negative cell line) expressing only Myc-p53 (first lane), and both Myc-p53 and His-Tip60. In the upper panel, the whole cell extracts were immuno-blotted with anti-Myc, anti-His-tag or anti-α-tublin antibodies. In the lower panel, the extracts were immuno-precipitated with anti-p53 Ac-K120 antibody (10E5) and the precipitates were immuno-blotted with anti-Myc antibody. Acetylation of p53 at K120 is dependent on

Tip60 and promoted by over-expression of His-Tip60.

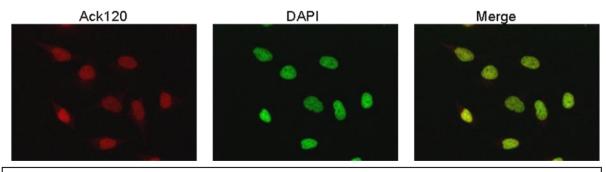


Fig.3. Immunofluorescence staining of p53 acetyl-K120 in nuclei of HeLa cells subjected to DNA damage.

HeLa cells were treated with 100 nM Doxorubicin for 24 hr, fixed with 4% paraformal dehyde overnight, permealized with 0.25% Triton X-100 in PBS for 10 min.

The antibody was used at 1/1,000 dilution

Nucleus (DNA) was stained with DAPI



References: This antibody has been used in the following publication.

Rokudai S et al. MOZ increases p53 acetylation and premature senescence through its complex formation with PML. Proc Natl Acad Sci U S A. 2013 Mar 5;110(10):3895-900.

PMID: <u>23431171</u>. **WB (human)**