Anti-p53 (acetyl K370) Antibody [SR40-09]

ET1602-38

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IP, FC
Molecular Wt:	Predicted band size: 53 kDa
Clone number:	SR40-09

Description: p53, a DNA-binding, oligomerization domain- and transcription activation domain-containing tumor suppressor, upregulates growth arrest and apoptosis-related genes in response to stress signals, thereby influencing programmed cell death, cell differentiation, and cell cycle control mechanisms. p53 localizes to the nucleus, yet can be chaperoned to the cytoplasm by the negative regulator, MDM2. MDM2 is an E3 ubiquitin ligase that is upregulated in the presence of active p53, where it poly-ubiquitinates p53 for proteasome targeting. p53 fluctuates between latent and active DNA-binding conformations and is differentially activated through posttranslational modifications, including phosphorylation and acetylation. Mutations in the DNA-binding domain (DBD) of p53, amino acids 110-286, can compromise energetically-favorable association with cis elements and are implicated in several human cancers.

Immunogen: Synthetic peptide within Human p53 aa 343-392 / 392 (acetyl K370).

Positive control: HeLa treated with 500ng/mL TSA for 4 hours cell lysate, NIH/3T3 treated with 500ng/mL TSA for 4 hours cell lysate, C6 treated with 1μM TSA for 18 hours cell lysate, Mouse brain tissue lysate, Rat brain tissue lysate, MCF-7, PANC-1, HeLa cells treated with 500ng/mL TSA for 4 hours.

Subcellular location: Cytoplasm, Nucleus, Endoplasmic reticulum, Mitochondrion matrix.

Database links: SwissProt: P04637 Human | P02340 Mouse | P10361 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:50
IF-Tissue	1:50
IP	Use at an assay dependent concentration.
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

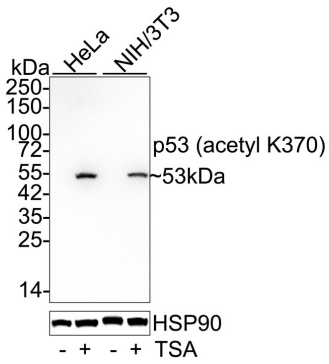

Fig1: Western blot analysis of p53 (acetyl K370) on different lysates with Rabbit anti-p53 (acetyl K370) antibody (ET1602-38) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 500ng/mL TSA for 4 hours cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: NIH/3T3 treated with 500ng/mL TSA for 4 hours cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 53 kDa
Observed band size: 53 kDa

Exposure time: 4 minutes; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1602-38) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

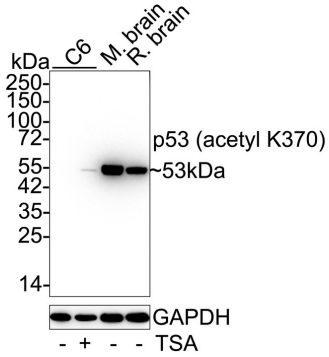

Fig2: Western blot analysis of p53 (acetyl K370) on different lysates with Rabbit anti-p53 (acetyl K370) antibody (ET1602-38) at 1/1,000 dilution.

Lane 1: C6 cell lysate (20 µg/Lane)
Lane 2: C6 treated with 1µM TSA for 18 hours cell lysate (20 µg/Lane)
Lane 3: Mouse brain tissue lysate (40 µg/Lane)
Lane 4: Rat brain tissue lysate (40 µg/Lane)

Predicted band size: 53 kDa
Observed band size: 53 kDa

Exposure time: 40 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1602-38) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

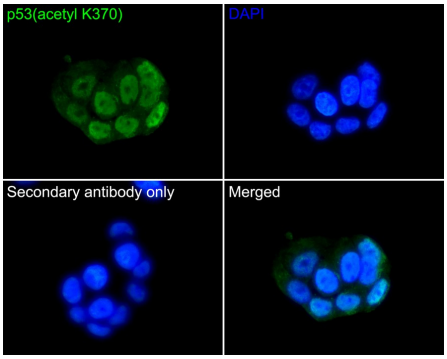

Fig3: Immunocytochemistry analysis of MCF-7 cells labeling p53 (acetyl K370) with Rabbit anti-p53 (acetyl K370) antibody (ET1602-38) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-p53 (acetyl K370) antibody (ET1602-38) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

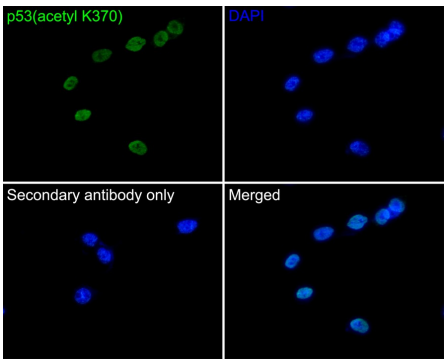

Fig4: Immunocytochemistry analysis of PANC-1 cells labeling p53 (acetyl K370) with Rabbit anti-p53 (acetyl K370) antibody (ET1602-38) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-p53 (acetyl K370) antibody (ET1602-38) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

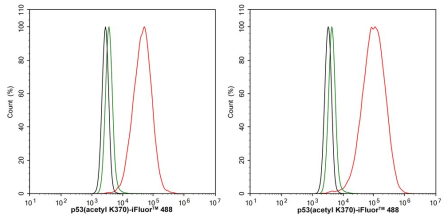

Fig5: Flow cytometric analysis of HeLa cells treated with 500ng/mL TSA for 4 hours labeling p53 (acetyl K370).

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1602-38, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Trabucco, S.E. et al. 2015. Inhibition of bromodomain proteins for the treatment of human diffuse large B-cell lymphoma. Clinical cancer research : an official journal of the American Association for Cancer Research. 21: 113-22.
2. Yang Zhang, et al. 2015. MicroRNA-520g confers drug resistance by regulating p21 expression in colorectal cancer. JBC. 4: 12.