Anti-p53 Antibody [SY010-6]

ET1605-16

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 53 kDa
Clone number:	SY010-6

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 N-terminal human p53.

Positive control: HT-29 cell lysate, A431 cell lysate, MDA-MB-231 cell lysate, HEK-293 cell lysate, MDA-MB-468 cell lysate, A431, human colon cancer tissue.

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

Database links: SwissProt: P04637 Human

Recommended Dilutions:

WB	1:1,000-1:5,000
IF-Cell	1:100
IF-Tissue	1:50-1:200
IHC-P	1:1,000
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

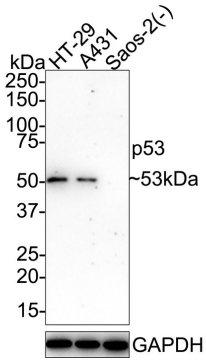

Fig1: Western blot analysis of p53 on different lysates with Rabbit anti-p53 antibody (ET1605-16) at 1/5,000 dilution.

Lane 1: HT-29 cell lysate
Lane 2: A431 cell lysate
Lane 3: Saos-2 cell lysate (negative)

Lysates/proteins at 15 µg/Lane.

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

Exposure time: 5 minutes;

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 (ET1605-16) at 1/5,000 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.

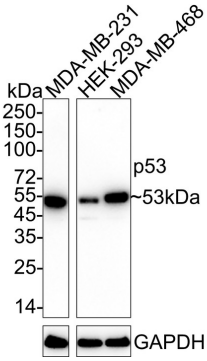

Fig2: Western blot analysis of p53 on different lysates with Rabbit anti-p53 antibody (ET1605-16) at 1/1,000 dilution.

Lane 1: MDA-MB-231 cell lysate
Lane 2: HEK-293 cell lysate
Lane 3: MDA-MB-468 cell lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 1 minute; 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 (ET1605-16) 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.

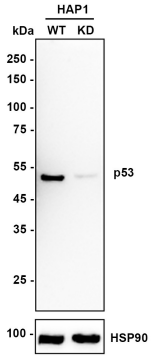

Fig3: Western blot analysis of p53 on different lysates with Rabbit anti-p53 antibody (ET1605-16) at 1/5,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-p53 KD cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 2 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 (ET1605-16) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

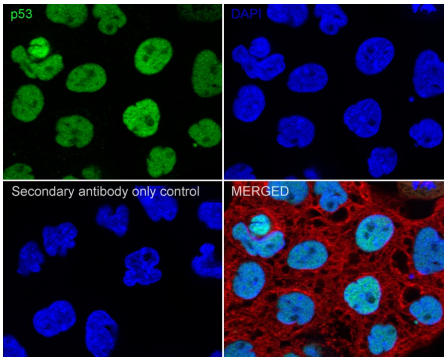

Fig4: Immunocytochemistry analysis of A431 cells labeling p53 with Rabbit anti-p53 antibody (ET1605-16) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-p53 antibody (ET1605-16) at 1/100 dilution in 1% BSA in PBST 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.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

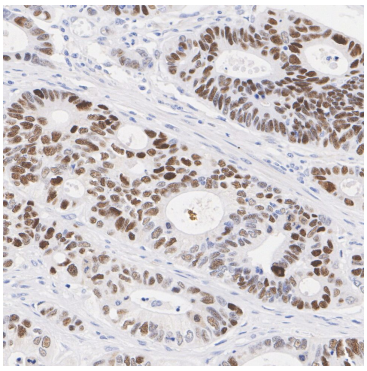

Fig5: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-p53 antibody (ET1605-16) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1605-16) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

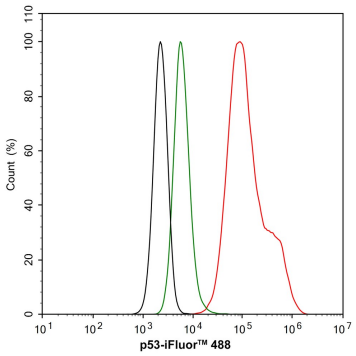

Fig6: Flow cytometric analysis of A431 cells labeling p53.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1605-16, 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. Yang K et al. Effect of PLCe gene silencing on inhibiting the cancerous transformation of ulcerative colitis. Exp Ther Med 12:422-426 (2016).
2. Ambade A et al. Alcoholic hepatitis accelerates early hepatobiliary cancer by increasing stemness and miR-122-mediated HIF-1a activation. Sci Rep 6:21340 (2016).