

# Anti-Phospho-p53 (S33) Antibody [JE41-97]

## HA722703



|                            |   |
|----------------------------|---|
| <b>Product Type:</b>       | Recombinant Rabbit monoclonal IgG, primary antibodies |
| <b>Species reactivity:</b> | Human, Mouse, Rat                                     |
| <b>Applications:</b>       | WB, IF-Cell, IHC-P                                    |
| <b>Molecular Wt:</b>       | Predicted band size: 53 kDa                           |
| <b>Clone number:</b>       | JE41-97   |

|                               |  |
|-------------------------------|--|
| <b>Description:</b>           | p53, also known as Tumor protein P53, cellular tumor antigen p53 (UniProt name), or transformation-related protein 53 (TRP53) is a regulatory protein that is often mutated in human cancers. The p53 proteins (originally thought to be, and often spoken of as, a single protein) are crucial in vertebrates, where they prevent cancer formation. As such, p53 has been described as "the guardian of the genome" because of its role in conserving stability by preventing genome mutation. Hence TP53 is classified as a tumor suppressor gene. The TP53 gene is the most frequently mutated gene (>50%) in human cancer, indicating that the TP53 gene plays a crucial role in preventing cancer formation. TP53 gene encodes proteins that bind to DNA and regulate gene expression to prevent mutations of the genome. In addition to the full-length protein, the human TP53 gene encodes at least 12 protein isoforms. |
| <b>Immunogen:</b>             | Synthetic phospho-peptide corresponding to residues surrounding Ser33 of Human p53 aa 1-50 / 393.  |
| <b>Positive control:</b>      | HT-29 cell lysate, HT-29 treated with 100ng/mL Nocodazole for 18 hours cell lysate, HT-29 cells treated with 100ng/mL Nocodazole for 18 hours, mouse liver tissue, human breast cancer tissue, human colon cancer tissue, human colon tissue, rat liver tissue.  |
| <b>Subcellular location:</b>  | Cytoplasm, Nucleus, PML body, Endoplasmic reticulum, Mitochondrion matrix, cytoskeleton, microtubule organizing center, centrosome.  |
| <b>Database links:</b>        | SwissProt: P04637 Human   P02340 Mouse   P10361 Rat  |
| <b>Recommended Dilutions:</b> |  |
| <b>WB</b>                     | 1:1,000  |
| <b>IF-Cell</b>                | 1:100  |
| <b>IHC-P</b>                  | 1:200  |
| <b>Storage Buffer:</b>        | 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.  |
| <b>Storage Instruction:</b>   | Store at +4°C after thawing. Aliquot store at -20°C. Avoid repeated freeze / thaw cycles.  |
| <b>Purity:</b>                | Protein A affinity purified.   |

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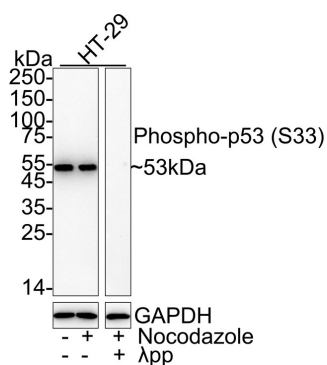
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## Images



**Fig1:** Western blot analysis of Phospho-p53 (S33) on different lysates with Rabbit anti-Phospho-p53 (S33) antibody (HA722703) at 1/1,000 dilution.

Lane 1: HT-29 cell lysate

Lane 2: HT-29 treated with 100ng/mL Nocodazole for 18 hours cell lysate

Lane 3: HT-29 treated with 100ng/mL Nocodazole for 18 hours cell lysate, then the membrane treated with  $\lambda$ pp for 1 hour

Lysates/proteins at 15  $\mu$ g/Lane.

Predicted band size: 53 kDa

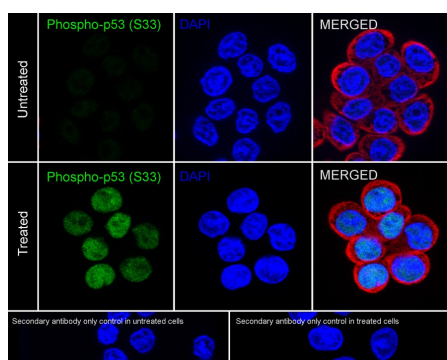
Observed band size: 53 kDa

Exposure time: 3 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 (HA722703) at 1/1,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

**Fig2:** Immunocytochemistry analysis of HT-29 cells treated with 100ng/mL Nocodazole for 18 hours labeling Phospho-p53 (S33) with Rabbit anti-Phospho-p53 (S33) antibody (HA722703) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-Phospho-p53 (S33) antibody (HA722703) at 1/100 dilution in 1% BSA in PBST overnight at 4°C. 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 (M1305-2, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

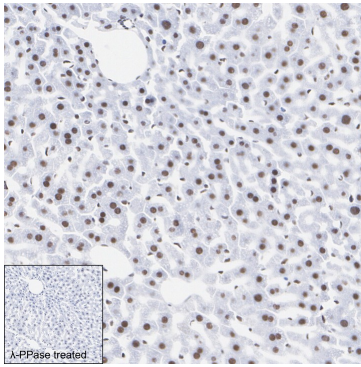
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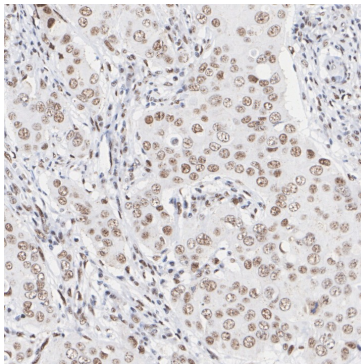
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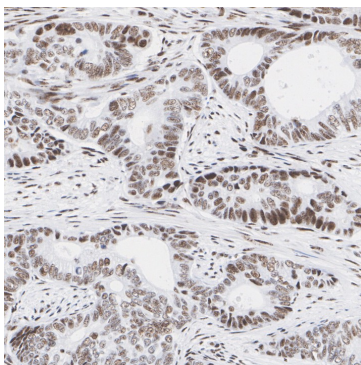
**Fig3:** Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-Phospho-p53 (S33) antibody (HA722703) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA722703) at 1/200 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.



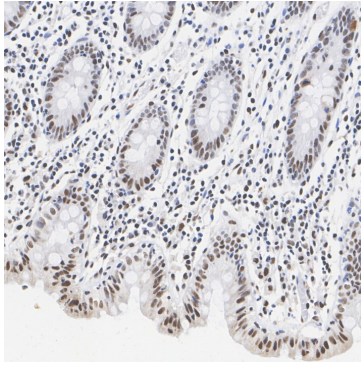
**Fig4:** Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-Phospho-p53 (S33) antibody (HA722703) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA722703) at 1/200 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.



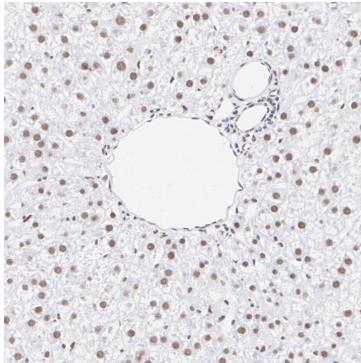
**Fig5:** Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-Phospho-p53 (S33) antibody (HA722703) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA722703) at 1/200 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:** Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-Phospho-p53 (S33) antibody (HA722703) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA722703) at 1/200 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.



**Fig7:** Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-Phospho-p53 (S33) antibody (HA722703) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA722703) at 1/200 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.

**Note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

### Background References

1. Hassin O et al. Drugging p53 in cancer: one protein, many targets. *Nat Rev Drug Discov.* 2023 Feb
2. Hu J et al. Targeting mutant p53 for cancer therapy: direct and indirect strategies. *J Hematol Oncol.* 2021 Sep

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