



Product Specification Sheet

p53 antibody

Cat # HP5311-A
Cat # HP5311-C

Rabbit anti-Human p53 antibody
Recombinant Human p53 protein for Western blotting

SIZE: 100 µg
SIZE: 100 µl

p53 (tumor suppressor protein), is a sequence specific transcription factor that is activated by cellular stress. p53 mediates cell cycle arrest or apoptosis in response to DNA damage or starvation for pyrimidine nucleotides. p53 is upregulated in response to stress signals and stimulated to activate transcription of specific genes, resulting in expression of p21waf1 and other proteins involved in G1 or G2/M arrest. The structure of p53 comprises an N-terminal transactivation domain, a central DNA-binding domain, an oligomerisation domain, and a C-terminal regulatory domain. There are various phosphorylation sites on p53, of which the phosphorylation at Ser15 is important for p53 activation and stabilization. p53 has been characterized to play a role in blocking the proliferative action of damaged cells and act as an anticancer agent. Phosphorylation of Ser392 in p53 has been shown to associate with the formation of human tumors. In addition, p53 has also been linked to the effects of aging and oxidative stress and an increase in p53 has been linked to deficits in LTP (Long Term Potentiation) in learning and memory. p53 is found in very low levels in normal cells, however, in a variety of transformed cell lines, it is expressed in high amounts, and believed to contribute to transformation and malignancy.

Source of Antigen and Antibodies

Uniprot: P04637

Host: Rabbit

Clonality: Polyclonal

Immunogen: Full length recombinant Human p53

Purification: Ammonium sulfate followed by protein affinity purification

Species Reactivity: Human

Cross reactivity: Full length p53 has >95% homology with Non-human primates and roughly 80% with Horse, Pig, Cat, Dog, Bovine, and Sheep. Due to the high degree of homology, it is expected to react with the above species although it has only been confirmed for in-house for Human.

Alternative names: Tumor suppressor p53

Subcellular Location: Nucleus

Recommended Secondary Antibody: Goat anti-Rabbit IgG-HRP (ADI cat#20320)

Form & Storage of Antibodies

Affinity pure IgG Solution

Concentration: 0.5 mg/ml Volume: 200 µl
Supplied in PBS, pH 7.4 + 0.1% BSA
The antibody can be made available carrier free or conjugated to HRP, Biotin, or FITC on request

Lyophilized powder

Lyophilized from a formulation containing PBS, pH 7.4 +3% Trehalose. Reconstitute in 200 µl PBS, 0.05% tween-20, 0.1% BSA, and a preservative to 0.5 mg/ml.

Storage:

Short-term: 4°C for 1 month

Long-term: at -20°C or below in suitable aliquots after reconstitution for 1 year. Do not expose to multiple freeze/thaw cycles or store working, diluted solutions. Glycerol may be added to a final concentration of 50% and antibodies can be stored un-aliquoted at -20°C.

Recommended Usage

ELISA: Assay dependent concentration. Typically, between 0.1-2.0 µg/ml for capture/detection antibodies. Request the carrier free or conjugated antibody.

Western Blotting: 0.5-1.0 µg/ml
Observed band size: 53 kDa

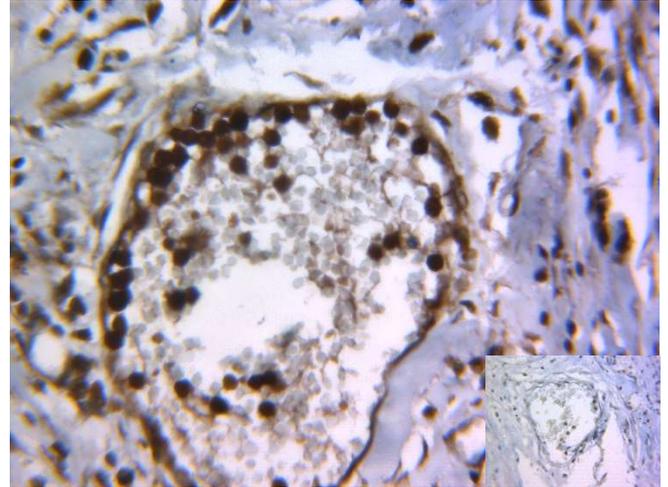
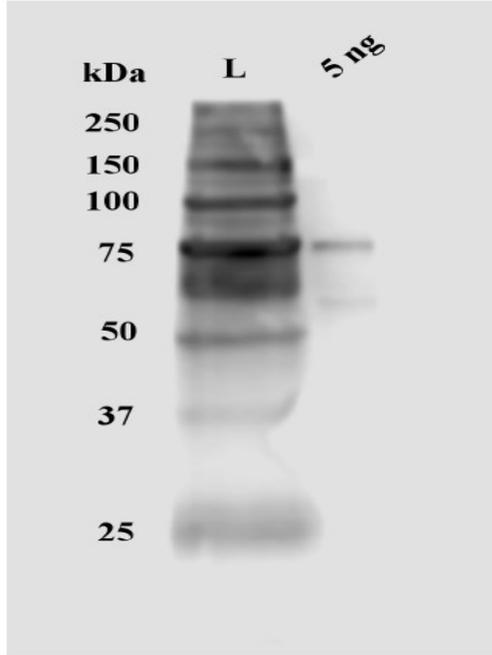
IHC-P: 1-10 µg/ml. QC tested using 10 mM sodium citrate, pH 6 antigen retrieval buffer. The antibody may work better with no retrieval or alternative retrieval solutions.

The above concentrations are a *suggestion*, user's must optimize their assay based on their own conditions. The antibody may work in other applications such as Immunocytochemistry or IP. These methods have not been tested by ADI.

**This product is for In vitro research use only.*

Related materials available from ADI

Catalog#	Description
BCL11-A	Rabbit Anti-Human BCL-2 antibody
BCL21-A	Rabbit Anti-Mouse BCL-2 antibody
BCL2-C	Recombinant BCL-2 control for Western blotting
AKT1-A	Rabbit anti-Human AKT1 antibody
AKT1-C	Recombinant AKT1 control for Western blotting
CASP3-A	Rabbit anti-Mouse Capase 3 antibody
HP5311-A	190728IA



Western blotting: 5 ng of recombinant p53 (**HP5311-C**) was heated for 5 minutes at 95°C then electrophoretically separated on a 4-20% SDS-PAGE gel. The gel was run at 100V for ~1 hour and 30 minutes then transferred to a 0.2 µm nitrocellulose membrane using the 'Mixed MW' settings on a Transblot Turbo (Biorad). The blot was blocked for 1 hour at room temperature with 1% Casein. **HP5311-A** was diluted with TBST+0.1% BSA to 1 µg/ml and incubated overnight at 4°C. The blot was washed with TBS-T 3 times for 5 minutes each. Goat anti-rabbit IgG HRP (**ADI cat#20320**) was diluted in TBST+0.1% BSA at a 1:1,000 dilution (500 ng/ml) then incubated for 1 hour at room temperature. The blot was washed 3 times with TBS-T for 5 minutes each. The blot was then incubated with regular ECL substrate for 1 minute and imaged on a CCD imaging system (C-DiGit, LI-COR).

Immunohistochemistry: FFPE Human Colon carcinoma slides were heated for 20 minutes at 60°C then deparaffinized. Antigen retrieval was performed for 10 minutes at 95°C in a microwave using 10 mM pH 6, sodium citrate buffer. The slide was then cooled for 20 minutes at room temperature before being blocked for 30 minutes with 2.5% normal goat serum. **HP5311-A** was diluted to 5 µg/ml in TBST+0.1% BSA and incubated overnight at 4°C (Inset represents recombinant p53 absorbed antibody incubation). The slides were then washed twice and incubated with 3% hydrogen peroxide for 10 minutes to quench endogenous peroxidase. The slide was washed then incubated with Goat anti-Rabbit IgG HRP polymer detection reagent for 30 minutes at room temperature. The slide was washed twice, incubated with DAB for 3 minutes, washed with distilled water, then counterstained for 1 minute with Gil's II Hematoxylin before being cover-slipped.

HP5311-C: Contains a recombinant *E.coli* expressed full length Human p53 protein at a concentration of 1 ng/µl in Laemmli buffer (62.5 mM Tris-HCL, pH 6.8, 2% SDS, 10% glycerol, 5% BME, and 0.002% bromphenol blue). Heat for 5 minutes at 95°C then load 1-5 µl. Store at -20°C in suitable size aliquots, do not expose to multiple freeze/thaw cycles.
Note: Due to the addition of a GST tag, the control will appear larger than native protein.