p53 Antibody

Rabbit Polyclonal

Antigen Affinity Purified

Catalog No. A300-247A

Lot No. A300-247A-2

Protein ID P04637

GeneID 7157

APPLICATIONS	WB, IP, IHC, ChIP-Seq, F
SPECIES REACTIVITY	Human
AMOUNT	100 µl
CONCENTRATION	1000 µg/ml
STORAGE/SHELF LIFE	2 - 8° C / 1 year from date of receipt
PHYSICAL STATE	Liquid
BUFFER	Tris-citrate/phosphate buffer, pH 7 to 8 containing 0.09% Sodium Azide
ISOTYPE	IgG
ORIGIN	USA
PRODUCTION PROCEDURES	Antibody was affinity purified using an epitope specific to p53 immobilized on solid support. The epitope recognized by A300-247A maps to a region between residues 50 and 100 of human tumor protein p53 using the numbering given in SwissProt entry P04637 (GeneID 7157). Immunoglobulin concentration was determined by extinction coefficient: absorbance at 280 nm of 1.4 equals 1.0 mg of IgG.
APPLICATIONS	Centrifuge tube to remove product from lid. Optimal working dilutions should be determined experimentally by the investigator. Prepare working dilution immediately before use.

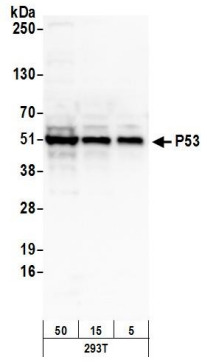
Western Blot	1:2,000 - 1:10,000
Immunoprecipitation	2 - 5 µg/mg lysate
Immunohistochemistry	1:1,000 - 1:5,000. Epitope retrieval with citrate buffer pH 6.0 is recommended for FFPE tissue sections.
ChIP-Seq	4 µg/30 µg chromatin
Flow Cytometry	1.5 µg per 1 X 10^6 cells in a 150 µl volume

APPLICATION NOTES	Western blot of immunoprecipitates performed using Normal Pig Serum (Cat. No. S100-020), Goat anti-Rabbit Light Chain HRP Conjugate (Cat. No. A120-113P) and 4-20% SDS-PAGE (link to IP-western blot protocol in Additional Info section below). Western blot of lysates performed using standard western blot reagents and 4-20% SDS-PAGE.
IHC HUMAN CONTROLS	Skin Squamous Cell Carcinoma
ADDITIONAL INFO	https://www.bethyl.com/product/A300-247A Use the link above to view SDS, a current list of citations, and other product specific information. IP-western blot protocol: https://www.bethyl.com/content/protocol_IP_WB

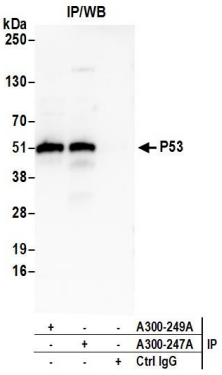
This document certifies that this product has met all of the quality control standards defined by Bethyl Laboratories, Inc.

Eric McIntush, PhD | Chief Scientific Officer

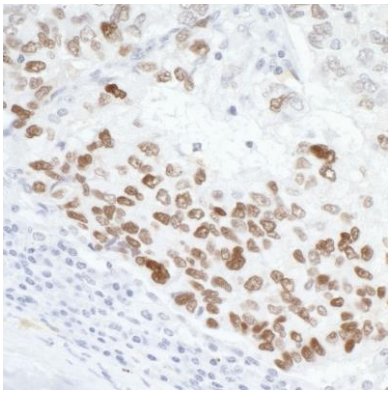
Date: June 21, 2019



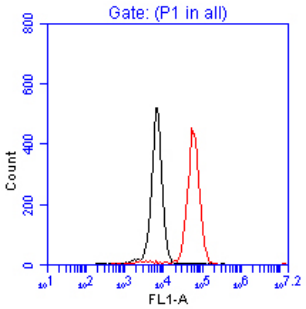
Detection of human P53 by western blot. *Samples:* Whole cell lysate (5, 15 and 50 µg) from HEK293T cells prepared using NETN lysis buffer. *Antibody:* Affinity purified rabbit anti-P53 antibody A300-247A (lot A300-247A-2) used for WB at 0.1 µg/ml. *Detection:* Chemiluminescence with an exposure time of 30 seconds.



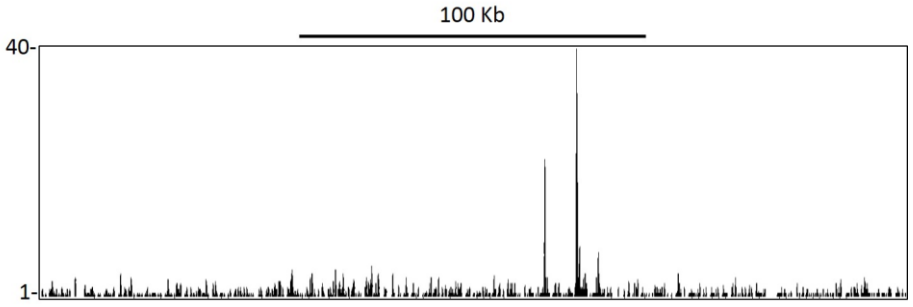
Detection of human P53 by western blot of immunoprecipitates. *Samples:* Whole cell lysate (1.0 mg per IP reaction; 20% of IP loaded) from HEK293T cells prepared using NETN lysis buffer. *Antibodies:* Affinity purified rabbit anti-P53 antibody A300-247A (lot A300-247A-2) used for IP at 3 µg per reaction. P53 was also immunoprecipitated by rabbit anti-P53 antibody A300-249A. For blotting immunoprecipitated P53, A300-247A was used at 1 µg/ml. *Detection:* Chemiluminescence with an exposure time of 30 seconds.



Detection of human p53 by immunohistochemistry. *Sample:* FFPE section of human lung cancer *Antibody:* Affinity purified rabbit anti- p53 (Cat. No. A300-247A Lot2) used at 1:5,000 (0.2µg/ml). *Detection:* DAB



Flow Cytometrical Analysis of p53 in Jurkat Cells. Jurkat cells were fixed in 1.5% PFA, and permeabilized in 90% Methanol. 1 X 10^6 cells were stained with 1.5 µg anti-KLH IgG control or anti-p53 [A300-247A] and secondary FITC-conjugated goat anti-rabbit (in a 150µl reaction). anti-KLH control IgG (black); anti-p53 (red).



Localization of p53 Binding Sites by ChIP-sequencing. Chromatin from MCF-7 was immunoprecipitated with anti-p53 antibody A300-247A and analyzed by DNA sequencing. The figure illustrates the peak distribution of p53 binding within a 250 Kb region of chromosome 6 as detected using anti-p53 A300-247A. ChIP-seq validation performed by Active Motif, Carlsbad, CA.