Human/Mouse/Rat p53 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1355

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat p53.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human p53 Asp7-Asp393 Accession # P04637
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 μm filtered solution in PBS.

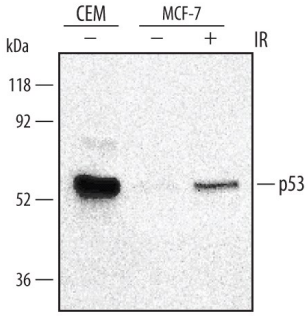
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.25 µg/mL	See Below
Chromatin Immunoprecipitation (ChIP)	5 µg/5 x 10^6 cells	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunoprecipitation	1-2 µg/500 µg cell lysate	CEM human T-lymphoblastoid cell line, see our available Western blot detection antibodies
Simple Western	2.5 µg/mL	See Below

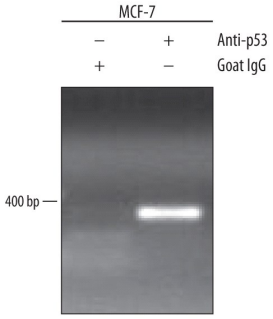
DATA

Western Blot



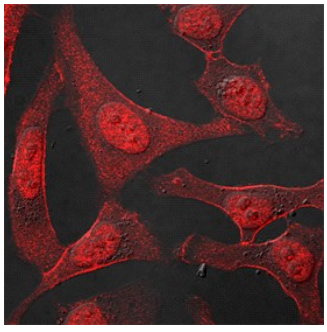
Detection of Human p53 by Western Blot. Western blot shows lysates of CEM human T-lymphoblastoid cell line and MCF-7 human breast cancer cell line were mock-treated (-) or exposed (+) to 10 Gy ionizing radiation (IR) and harvested after 1 hour. PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human/Mouse/Rat p53 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1355), followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). A specific band was detected for p53 at approximately 53 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Chromatin Immunoprecipitation (ChIP)



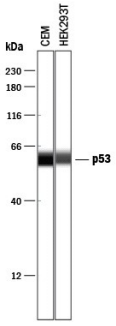
Detection of p53-regulated Genes by Chromatin Immunoprecipitation. MCF-7 human breast cancer cell line treated with 300 nM camptothecin overnight were fixed using formaldehyde, resuspended in lysis buffer, and sonicated to shear chromatin. p53/DNA complexes were immunoprecipitated using 5 µg Goat Anti-Human/Mouse/Rat p53 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1355) or control antibody (Catalog # AB-108-C) for 15 minutes in an ultrasonic bath, followed by Biotinylated Anti-Goat IgG Secondary Antibody (Catalog # BAF109). Immunocomplexes were captured using 50 µL of MagCellect Streptavidin Ferrofluid (Catalog # MAG999) and DNA was purified using chelating resin solution. The p21 promoter was detected by standard PCR.

Immunocytochemistry



p53 in HeLa Human Cell Line. p53 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Goat Anti-Human/Mouse/Rat p53 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1355) at 1.7 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Simple Western



Detection of Human p53 by Simple Western™. Simple Western lane view shows lysates of CEM human T-lymphoblastoid cell line and HEK293T human embryonic kidney cell line, loaded at 0.2 mg/mL. A specific band was detected for p53 at approximately 59 kDa (as indicated) using 2.5 µg/mL of Goat Anti-Human/Mouse/Rat p53 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1355) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

The p53 tumor suppressor protein is a multi-functional transcription factor that regulates cellular decisions regarding proliferation, cell cycle checkpoints, and apoptosis. The importance of p53 is underscored by its mutation in over 50% of human cancers. Mice that lack one or both copies of p53 also showed an increased incidence of tumors, which makes the p53 deficient mouse a model system for studying cancer generation and progression.