Human Phospho-p53 (S15) Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: AF1043

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human, monkey, and rat p53 when phosphorylated at S15 and mouse p53 when phosphorylated at S18.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	Phosphopeptide containing human p53 S15 site
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.

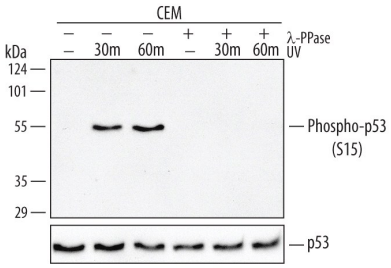
APPLICATIONS

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	0.2 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Immunoprecipitation	1 µg/500 µg cell lysate	CEM human T-lymphoblastoid cell line exposed to UV-C and MCF-7 human breast cancer cell line treated with camptothecin (CPT), see our available Western blot detection antibodies

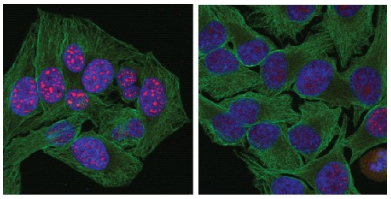
DATA

Western Blot



Detection of Human Phospho-p53 (S15) by Western Blot. Western blot shows lysates of CEM human T-lymphoblastoid cell line untreated or exposed to 100 J/m^2 UV-C for the indicated time. PVDF membrane was probed with 0.2 µg/mL Human Phospho-p53 (S15) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1043) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band for Phospho-p53 (S15) was detected at approximately 53 kDa (as indicated). The phospho-specificity of this antibody was supported by decreased labeling following treatment with 600 U λ-phosphatase (λ-PPase) for 1 hour. For additional reference, duplicate lysates were probed with 1:5000 dilution Human/Mouse/Rat p53 HRP-conjugated Antigen Affinity-purified Polyclonal Antibody (*lower panel*, Catalog # HAF1355). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



Treated **Untreated**

Phospho-p53 (S15) in HeLa Human Cell Line. p53 phosphorylated at S15 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line treated with UV light (left panel) or untreated (right panel) using Rabbit Anti-Human Phospho-p53 (S15) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1043) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to nuclei. Cells were co-stained using Rat Anti-Tubulin (Catalog # NB600-506, Novus Biologicals) and NorthernLights™ 493-conjugated Anti-Rat IgG Secondary Antibody (green; Catalog # NL015). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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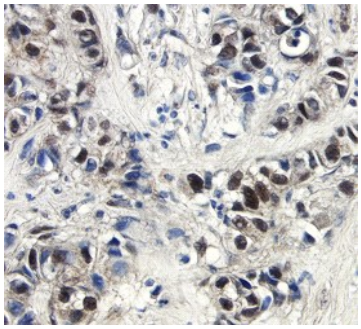
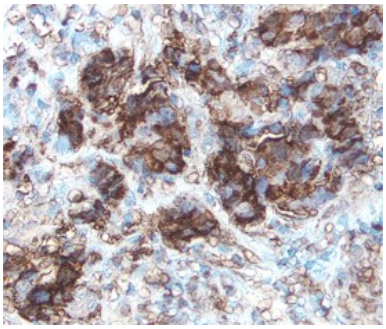
Immunohistochemistry



Phospho-p53 (S15) in Human Breast Cancer Tissue. p53 phosphorylated at S15 was detected in immersion fixed paraffin-embedded sections of human breast cancer tissue using Human Phospho-p53 (S15) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1043) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Rabbit HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS005) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of immersion fixed paraffin-embedded Tissue Sections.

Immunohistochemistry



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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.

BACKGROUND

The p53 tumor suppressor protein is a multi-functional transcription factor that regulates proliferation, cell cycle checkpoints, and apoptosis. The importance of p53 is underscored by its mutation in over 50% of human cancers. Human p53 is a 393 amino acid (aa) nuclear/cytoplasmic protein that contains an N-terminal activation domain, a specific DNA binding domain, and a C-terminal domain that mediates tetramer formation and regulates DNA binding. P53 is phosphorylated on Ser15 in response to irradiation, and this phosphorylation increases recruitment of the coactivator CREB-binding protein/p300. The ATM or ATR kinases can phosphorylate p53 at serine 15 (S15), which leads to cell cycle arrest. Serine 15 phosphorylation leads to p53 stabilization and enhances transactivation of p53 target genes.

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