Human Phospho-p53 (S15) Antibody

Monoclonal Mouse IgG_1 Clone # 261352

Catalog Number: MAB1839

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human p53 that is phosphorylated at S15 in Western blots. In Western blots, no cross-reactivity with human p53 that is unphosphorylated at S15 is detected.
Source	Monoclonal Mouse IgG_1 Clone # 261352
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Phosphopeptide containing the human p53 S15 site 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 either lyophilized or as a 0.2 µm filtered solution in PBS.

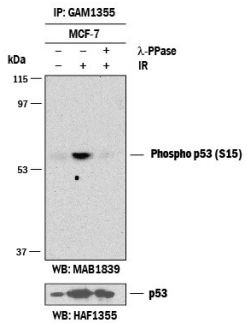
APPLICATIONS

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	1-2 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10^6 cells	See Below
CyTOF-reported	Brodie, T.M. *et al.* (2018) Cytometry Part A. **93**: 406. Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

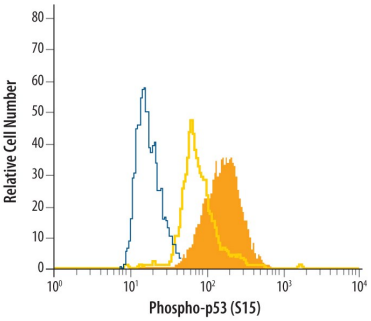
DATA

Western Blot



Detection of Human Phospho-p53 (S15) by Western Blot. Western blot shows p53 immunoprecipitated from lysates of MCF-7 human breast cancer cell line using Human/Mouse/Rat p53 Agarose-conjugated Antigen Affinity-purified Polyclonal Antibody (Catalog # GAF1355). MCF-7 cell line was untreated (-) or exposed (+) to 10 Gy ionizing radiation (IR) for 3 hours. PVDF membrane was probed with 1-2 µg/mL Mouse Anti-Human Phospho-p53 (S15) Monoclonal Antibody (Catalog # MAB1839) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). 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 the membrane was stripped and reprobed with 1:5000 dilution Human/Mouse/Rat p53 HRP-conjugated Antigen Affinity-purified Polyclonal Antibody HAF1355 (*lower panel*, Catalog # HAF1355) This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Intracellular Staining by Flow Cytometry



Detection of p53 in camptothecin-treated MCF-7 Human Cell Line by Flow Cytometry. MCF-7 human breast cancer cell line was unstimulated (light orange open histogram) or treated with 1 µM camphtothecin for 6 hours (dark orange filled histogram), then stained with Mouse Anti-Human Phospho-p53 (S15) Monoclonal Antibody (Catalog # MAB1839) or isotype control (Catalog # MAB002, blue open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG $F(ab')_2$ Secondary Antibody (Catalog # F0102B). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with saponin.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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 acts to enforce cell cycle checkpoints or signal apoptosis in cells that have incurred genotoxic damage. 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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