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### Certificate of Analysis

**Product:** Protein A Purified Murine Monoclonal Anti-p53

**Code:** 200-301-174

**Lot #:** 9832

**Size:** 100 µg

**Clone:** BP53.12

**Isotype:** IgG<sub>2a</sub> Kappa

**Physical State:** Liquid (sterile filtered)

**Antibody Concentration:** 1.0 mg/ml (by UV absorbance at 280 nm)

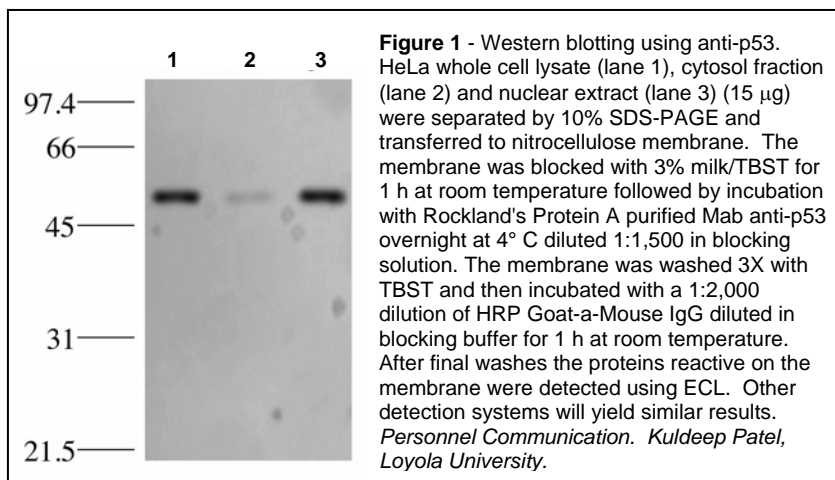
**Buffer:** 0.02 M Potassium Phosphate, 0.5 M Sodium Chloride, pH 7.2

**Stabilizer:** None

**Preservative:** 0.01% (w/v) Sodium Azide

**Storage Conditions:** Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use. Expiration date is one (1) year from date of opening.

**Background Information:** The p53 gene like the Rb gene, is a tumor suppressor gene, i.e., its activity stops the formation of tumors. If a person inherits only one functional copy of the p53 gene from their parents, they are predisposed to cancer and usually develop several independent tumors in a variety of tissues in early adulthood. This condition is rare, and is known as Li-Fraumeni syndrome. However, mutations in p53 are found in most tumor types, and so contribute to the complex network of molecular events leading to tumor formation. In most transformed and tumor cells the concentration of p53 is increased 5-1000 fold over the minute concentrations (1000 molecules/cell) in normal cells, principally due to the increased half-life (4 h) compared to that of the wild-type (20 min). The p53 gene has been mapped to chromosome 17. In the cell, p53 protein binds DNA, which in turn stimulates another gene to produce a protein called p21 that interacts with a cell division-stimulating protein (cdk2). When p21 is complexed with cdk2 the cell cannot pass through to the next stage of cell division. Mutant p53 can no longer bind DNA in an effective way, and as a consequence the p21 protein is not made available to act as the 'stop signal' for cell division. Thus cells divide uncontrollably, and form tumors. p53 localizes in the nucleus, but is detectable at the plasma membrane during mitosis and when certain mutations modulate cytoplasmic/nuclear distribution. Help with unraveling the molecular mechanisms of cancerous growth has come from the use of mice as



models for human cancer, in which powerful 'gene knockout' techniques can be used. The amount of information that exists on all aspects of p53 normal function and mutant expression in human cancers is now vast, reflecting its key role in the pathogenesis of human cancers. It is clear that p53 is just one component of a network of events that culminate in tumor formation.

#### Recommended Dilutions:

ELISA	1:2,000 - 1:10,000
WESTERN BLOT	1:500 - 1:2,000
ChIP	1 µg/µl 4° o/n
IMMUNOHISTOCHEMISTRY	1:50
OTHER APPLICATIONS	User Optimized

**Application Note(s):** This antibody is suitable for ChIP, flow cytometry, immunohistochemistry, immunoblotting and immunoprecipitation. p53 is the most commonly mutated gene in spontaneously occurring human cancers. Mutations arise with an average frequency of 70% but incidence varies from zero in carcinoid lung tumors to 97% in primary melanomas. High concentrations of p53 protein are transiently expressed in human epidermis and superficial dental fibroblasts following mild ultraviolet irradiation. This antibody reacts with an N-terminal epitope of the 53 kD gene product and this epitope is not destroyed by formalin-fixation and routine paraffin embedding. Microwaving is needed for optimal staining.

**Purity and Specificity:** This protein A purified mouse monoclonal antibody reacts specifically with p53 in human tissues and cell lines. The antibody recognizes a 53 kDa band corresponding to p53. Cross reactivity with p53 from other sources has not been determined.

**Immunogen:** This protein A purified monoclonal antibody was produced by repeated immunizations with recombinant human p53 protein.

**Hybridoma:** Produced by the fusion between BALB/c mouse splenocytes and mouse myeloma P3-X63/AG8.653 cells using conventional hybridoma technology.

#### Specific Reference(s):

Gurney, E. G. et al (1980) Monoclonal antibodies against simian virus 40 T antigens: evidence for distinct subclasses of large T antigen and for similarities among nonviral T antigens. *J Virol*, **34**(3):752-63.

Hollstein, M, et al. (1991) p53 mutations in human cancers. *Science*, **253**: 49-53.

Lane, D.P. (1992) p53, guardian of the genome. *Nature*, **358**(6381):15-16

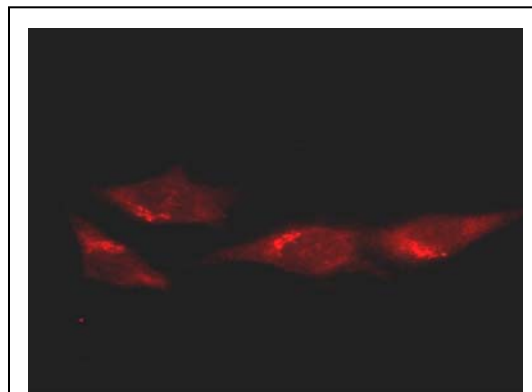
Vogelstein, B. and Kinzler, K.W. (1992) p53 function and dysfunction. *Cell*, **70**(4):523-6.

Donehower, L.A. and Bradley, A. (1993) The tumor suppressor p53. *Biochim Biophys Acta*, **1155**(2):181-205.

#### Relevant Link(s):

Structure of [p53](#) core domain

**Note:** This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 326, Gilbertsville, Pennsylvania, USA.



**Figure 2** - Immunofluorescence microscopy of HeLa cells using anti-p53. Rockland's Protein A purified Mab anti-p53 was used at a 1:100 dilution in 10% normal goat serum in PBS and reacted overnight at 4° C. After washes cells were incubated with a 1:500 dilution of AlexaFluor™594 Goat-a-Mouse IgG diluted in normal goat serum for 1 h at room temperature. *Personnel Communication. Kuldeep Patel, Loyola University.*