MONOCLONAL ANTIBODY p53 Protein

Cat. No.	Form	Quantity	Presentation
1407	Pre-diluted	6 ml	Ready-to-use

Clone DO-1

Isotype IgG2a (mouse)

Immunogen Recombinant human wild-type p53 protein (1).

Hybridoma Myeloma cell X 63 Ag8.653 x Balb/c mouse splenocytes.

Specificity DO-1 reacts with p53, an oncoprotein involved in cell cycle regulation (2,3). The antibody recognizes a fixative-resistant epitope on the N-terminal 45 amino acids 37 and 45. DO-1 reacts with both wild-type and mutant forms of P53. However, the normal protein has a very short half-life . Only the mutant form whose half-life is longer can be immunostained. This antibody recognizes monkey and bovine p53, but not mouse p53 (1,2).

Reactivity Normal tissues: The nuclei of some very rare non-neoplastic cells may immunostain with DO-1 monoclonal antibody in various normal human tissues (4-8).

Tumor tissues: Strong nuclear positivity is seen in different maligancies with an elevated level of p53 (2,4,6, 8-17).

Applications Studies indicate that p53 antibody may be useful as a cell proliferation marker in tumor cells and pre-malignant lesions on fixed-embedded and frozen tissue sections, as well as in cytological samples (smears/imprints/cytospins) (17-18).

Buffer 50 mM Tris-HCl, 0.15 M NaCl, pH 7.2 containing 1 mg/ml bovine serum albumin and 0.1% sodium azide. The buffer contains a green dye.

Recommended Procedures DO-1 antibody is ready for use on cytological samples and frozen sections or routinely fixed (methacarn, formalin, Bouin's, B5), paraffin-embedded tissue sections previously processed using heat treatment*(19).

1- Paraffin sections: Heat or enzymatic treatment may induce loss of adherence of tissue sections to glass slides. This may be overcome by using glass slides coated according to one of a) or b) procedures:

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a) Gelatin-Chrome Alum Dip Method (20):

2,5 g of gelatin and 2 g of KCr(SO4)2, 12H2O in 500 ml distilled water at 40-50°C.

Slides should be dipped into this solution for 1 second and allowed to dry at room temperature.

b) Aminoalkylsilane (APES): (21)

1 ml of 3-aminopropylriethoxysilane in 50 ml acetone.

Slides should be dipped into this solution for 20 seconds, then washed twice in aceton and twice in distilled water.

Tissue sections are floated onto slides in a waterbath at 37°C, prior to being dried overnight at 37°C or at room temperature for 48 hours.

c) Heat treatment:

10 mM Citrate Buffer, pH 6 (22)

stock solution A: 0.1M citric acid

stock solution B: 0.1M sodium citrate.

Store at 2-8°C.

9 ml of A + 41 ml of B, add distilled water to a final volume of 500 ml.

Heating of tissue sections: Slide processing in a microwave oven, burner heated water path or other heating equipment*.

Deparaffinized slides should be placed in a thermoresistant dish filled with citrate buffer. Run 3-5 cycles of 5 minutes each at 750 watts. Boiling is normally observed. Refill the dish with distilled water to replace evaporated water. Sections should not dry. To avoid bubble trapping between slides, it is recommended to leave at least 4 mm between slides.

Remove the dish from the oven and allow to cool down for 20 minutes at room temperature. Rinse slides in TBS buffer.

d) Trypsin treatment of sections prior to heating may enhance staining intensity (24, 25): sections should be treated with a Trypsin solution (0.1FIP-U per ml of Phosphate-Buffered Saline (PBS) or Tris Buffer Saline (TBS) at 37°C for 10-20 minutes. The reaction should be stopped in water.

2- Cytological samples and frozen sections do not require any heat treatment.

3- Process immunostaining according to previously described methods (23): the antibody should be incubated on tissue sections for 60 minutes at room temperature.

*Depending on the exact protocol followed, this step may require a license under U.S. Patent 5,244,787.

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