Monoclonal Mouse Anti-Human p53 Protein
Clone DO-7
Code No. M 7001
Lot 121, Edition 19.02.03

Intended use
For in vitro diagnostic use.
Monoclonal Mouse Anti-Human p53 Protein is intended for use in immunocytochemistry. The antibody labels wild-type and mutant-type p53 protein and is a useful tool for the identification of p53 accumulation in human neoplasias (1-3). Differential identification is aided by the results from a panel of antibodies. Interpretation must be made within the context of the patient’s clinical history and other diagnostic tests by a qualified pathologist.

Introduction
p53 is a nuclear phosphoprotein with a molecular mass of 53 kDa. Wild-type p53 protein is present in a wide variety of normal cells, but the protein has a very short half-life and thus is present in only minute amounts (1), generally below the detection level of immunocytochemical methods (4). Somatic mutation of the p53 gene is a very frequent event in the development of human neoplasia, and because mutant p53 proteins often are much more stable than wild-type p53 protein, the mutant p53 protein accumulates to a high level (1). As examples, p53 protein accumulation was observed in 76% of 212 human malignant lesions, including breast, colon and stomach carcinomas, melanoma, embryonal carcinoma of the testis, transitional carcinoma of the urinary bladder, uterine carcinoma and soft tissue sarcomas (5).
Wild-type p53 protein functions as a transcription factor, i.e. as a modulator which can turn crucial genes either on or off. It also inhibits DNA replication and is a check-point control molecule for progression of the cell cycle. Furthermore, p53 protein is involved in the regulation of apoptosis (2). In transfection assays, wild-type p53 behaves as a tumour suppressor, while mutant p53 behaves as a dominant transforming oncogene (1).

Reagent provided
Monoclonal mouse antibody provided in liquid form as cell culture supernatant dialysed against 0.05 mol/L Tris/HCl, pH 7.2, and containing 15 mmol/L NaN3.

Clone: DO-7 (1). Isotype: IgG2b, kappa.
Mouse IgG concentration: 335 mg/L. Total protein concentration: 20.4 g/L.

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

Specificity
SDS-PAGE analysis of immunoprecipitates formed between lysate of the BT474 breast cancer cell line and the antibody shows reaction with a 53 kDa protein corresponding to p53 (1).
In Western blotting of lysate of the A431 human vulval carcinoma cell line, the antibody labels a 53 kDa band, corresponding to the mutant-type p53, which is expressed by A431. The epitope recognized by the antibody is located between the N-terminal amino acids 1 and 46 and possibly between amino acids 37 and 45 of the human p53 protein (1).
In immunocytochemistry the antibody labels mutant-type p53 in the A431 cell line and wild-type p53 in the SVK14 cell line (SV40-transformed keratinocyte line) (1).

Precautions
1. For in vitro diagnostic use.
2. This product contains sodium azide (NaN3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.

Storage
Store at 2-8 ºC. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the user must verify the conditions. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact our Technical Services.

Specimen preparation
Paraffin sections: The antibody can be used for labelling paraffin-embedded tissue sections fixed in formalin or methacarn (1). Pre-treatment of tissues with heat-induced epitope retrieval is required. For tissues fixed in formalin, optimal results are obtained with DakoCytomation Target Retrieval Solution, High pH, code No. S 3308, or 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0. Less optimal results are obtained with DakoCytomation Target Retrieval Solution, code No. S 1700, or 10 mmol/L citrate buffer, pH 6.0. Pre-treatment of tissues with proteinase K was found inefficient. The tissue sections should not dry out during the treatment or during the following immunocytochemical staining procedure.
Frozen sections and cell preparations: The antibody can be used for labelling acetone-fixed, frozen sections and acetone-fixed, cultured cells (1).

(2)
**Staining procedure**

**Dilution:** Monoclonal Mouse Anti-Human p53 Protein, code No. M 7001, may be used at a dilution range of 1:25-1:50 when applied on formalin-fixed, paraffin-embedded sections of breast carcinoma and epidermoid carcinoma cell line and using 20 minutes heat-induced epitope retrieval in DakoCytomation Target Retrieval solution. High pH, code No. S 3308, and 30 minutes incubation at room temperature with the primary antibody. Optimal conditions may vary depending on specimen and preparation method, and should be determined by each individual laboratory. The recommended negative control is DakoCytomation Mouse IgG2b, code No. X 0944, diluted to the same mouse IgG concentration as the primary antibody.

**Visualization:** DAKO LSAB™+/HRP kit, code No. K 0679, and DAKO EnVision™+/HRP kits, code Nos. K 4004 and K 4006, are recommended. For frozen sections and cell preparations, the DakoCytomation APAAP kit, code No. K 0670, is a good alternative if endogenous peroxidase staining is a concern. Follow the procedure enclosed with the selected visualization kit.

**Automation:** The antibody is well-suited for immunocytochemical staining using automated platforms, such as the DakoCytomation Autostainer.

**Performance characteristics**

Cells labelled by the antibody generally display a nuclear staining pattern, but cytoplasmic staining has been reported in some cases (6).

**Normal tissues:** In normal and reactive mesothelium the antibody labelled 0/40 cases, and in 27 mesotheliomas, normal cells, e.g. fibroblasts and endothelial cells were negative (3).

**Abnormal tissues:** In follicular lymphoma an increasing accumulation of p53 in centroblasts is observed with morphological progression resulting in 1/16 cases of grade I, 10/21 cases of grade II, and 6/6 cases of grade III being positive (4). In mesotheliomas the antibody labelled 7/26 cases of epithelial type (1 to 25% positive cells), 1/7 cases of mixed type (25 to 50% positive cells), and 1/3 cases of mesenchymal type (more than 75% positive cells) (3). In Hodgkin’s lymphoma 65% or more show positive labelling for p53, whereas around 50% of non-Hodgkin’s lymphomas are positive (2).

**References**


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