Technical Data Sheet

FITC Mouse Anti-p53 Antibody Set

Product Information

Material Number:	**554298**
Size:	100 tests
Reactivity:	QC Testing: Human Tested in Development: Monkey, Cow
Component:	**51-15804X**
Description:	FITC Mouse Anti-Human p53
Size:	100 tests (1 ea)
Vol. per Test:	20 µl
Clone Name:	DO-7
Isotype:	Mouse IgG2b
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.
Component:	**51-66374X**
Description:	FITC Mouse IgG2b Isotype Control
Size:	100 tests (1 ea)
Vol. per Test:	20 µl
Clone Name:	27-35
Isotype:	Mouse (C.SW) IgG2b, κ
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

p53 is a 53 kD nuclear phosphoprotein that acts as a tumor suppressor protein, and is involved in inhibiting cell proliferation when DNA damage occurs. The gene for p53 is the most commonly mutated gene yet identified in human cancers. Missense mutations occur in tumors of the colon, lung, breast, ovary, bladder and several other organs. The mutant p53 is overexpressed in a variety of transformed cells and wild-type p53 forms specific complexes with several viral oncogenes including SV40 large T, E1B from adenovirus, and E6 from human papilloma virus. Wild type p53 plays a role as a checkpoint protein for DNA damage during the G1/S-phase of the cell cycle. However, it is still unclear, whether point mutated forms of p53 are simple null mutants and/or dominant negatively acting proteins.

DO-7 recognizes human wildtype and mutant p53. It cross-reacts with bovine p53 but does not cross-react with mouse or rat p53. DO-7 recognizes an epitope between amino acids 1-45 of the known forms of human p53. Human recombinant p53 protein was used as immunogen. Wildtype 53 proteins have a very short half-life and are usually not detectable with monoclonal antibodies in normal tissues. Clone DO-7 was initially characterized by immunoprecipitation, western blot analysis, immunohistochemistry of frozen and formalin-fixed paraffin-embedded tissue sections, and flow cytometric analysis.

Clone 27-35 is an isotype control monoclonal antibody specific for hapten dansyl (5-[dimethylamino] naphthalene-1-sulfonyl). This hapten is not expressed on human cells or human cell lines. The DO-7 and 27-35 FITC conjugates are matched in F/P ratios. The optimum F/P ratio was experimentally determined by flow cytometric analysis.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.

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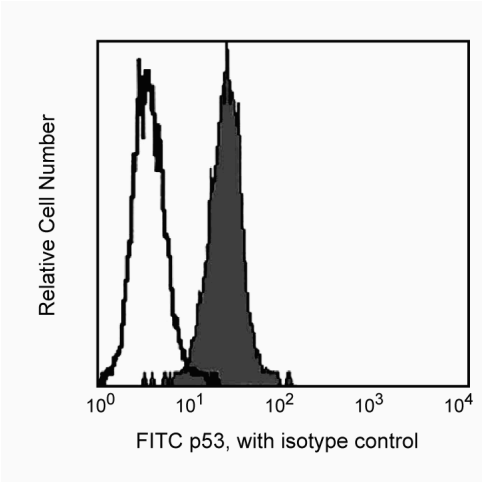

Profile of permeabilized HT-29 colon adenocarcinoma cells analyzed on a FACScan™ (BDIS, San Jose, CA). *Cells were stained with anti-human p53-FITC (clone DO-7) or with an IgG2b isotype control.*

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Positive control cell lines include SK-BR-3 human breast carcinoma cells (ATCC HTB 30), HT-29 colon adenocarcinoma (ATCC HTB 38) and A431 human vulval carcinoma cells (ATCC CRL 1555). MCF-7 human breast carcinoma cells (ATCC HTB 22) are suggested as a negative control.

Product Notices

1. This antibody has been optimized and preassayed with its matched isotype control to be used at the recommended volume of 20 ul/test. Titration of the reagents or substituting with other (non-matched) isotype control is NOT recommended.
2. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Baas IO, Mulder JW, Offerhaus GJ, Vogelstein B, Hamilton SR. An evaluation of six antibodies for immunohistochemistry of mutant p53 gene product in archival colorectal neoplasms. *J Pathol.* 1994; 172(1):5-12. (Clone-specific: Immunohistochemistry)

Bonsing BA, Corver WE, Gorsira MC, et al. Specificity of seven monoclonal antibodies against p53 evaluated with Western blotting, immunohistochemistry, confocal laser scanning microscopy, and flow cytometry. *Cytometry.* 1997; 28(1):11-24. (Clone-specific: Flow cytometry)

Cripps KJ, Purdie CA, Carder PJ, et al. A study of stabilisation of p53 protein versus point mutation in colorectal carcinoma. *Oncogene.* 1994; 9(9):2739-2743. (Clone-specific: Immunohistochemistry)

Jacquemier J, Moles JP, Penault-Llorca F, et al. p53 immunohistochemical analysis in breast cancer with four monoclonal antibodies: comparison of staining and PCR-SSCP results. *Br J Cancer.* 1994; 69(5):846-852. (Clone-specific: Immunohistochemistry)

Lambkin HA, Mothersill CM, Kelehan P. Variations in immunohistochemical detection of p53 protein overexpression in cervical carcinomas with different antibodies and methods of detection. *J Pathol.* 1994; 172(1):13-18. (Clone-specific: Immunohistochemistry)

Vogelstein B. Cancer. A deadly inheritance. *Nature.* 1990; 348(6303):681-682. (Biology)

Vojtesek B, Bartek J, Midgley CA, Lane DP. An immunochemical analysis of the human nuclear phosphoprotein p53. New monoclonal antibodies and epitope mapping using recombinant p53. *J Immunol Methods.* 1992; 151(1-2):237-244. (Clone-specific: Immunohistochemistry, Immunoprecipitation, Western blot)

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