BD Pharmingen™ Technical Data Sheet

p53 **PURIFIED MOUSE ANTI-HUMAN p53 MONOCLONAL ANTIBODY FOR IMMUNOHISTOCHEMISTRY (IHC).**

PRODUCT INFORMATION

Catalog Number:	**550832** (Was: 75571E), **1 ml**
Clone:	G59-12
Antibody Isotype:	Mouse IgG_1, κ
Storage Buffer:	Purified IHC diluent containing serum* 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.[1] 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 wildtype p53 forms specific complexes with several viral oncogenes including SV40 large T, E1B from adenovirus, and E6 from human papilloma virus. Wildtype p53 plays a role as a checkpoint protein for DNA damage during the G1/S-phase of the cell cycle.[2] G59-12 recognizes mutant and wild type human, rat and mouse p53 tumor suppressor protein.[3,4] Recombinant full-length human p53 expressed in the baculovirus expression system (BEVS) was used as immunogen.

PREPARATION AND STORAGE

The antibody is purified from tissue culture supernatant by Protein G affinity chromatography. Store at 4°C.

APPLICATION NOTES

Immunohistochemistry: The anti-p53 antibody is tested for immunohistochemical staining of acetone-fixed frozen sections and formalin-fixed paraffin sections. For paraffin sections microwave oven pretreatment with BD Retrievagen A (pH 6.0) (Cat. No. 550524/7539KK) is required. Tissues tested were human cancerous tissue. The normal concentrations of p53 protein is generally below the detection level of IHC methods, however in a variety of tumors the p53 is mutated and this accumulation of mutant p53 protein can be detected in the cell nuclei. The isotype control recommended for use with this antibody is purified mouse IgG_1 (Cat. No. 550878/75761E). For optimal indirect immunohistochemical staining, anti-p53 antibody should be titrated (1-10 to 1-50 dilution) and visualized via a three-step staining procedure in combination with polyclonal, biotin conjugated anti-mouse Igs (multiple adsorbed) (Cat. No. 550337/74462E) as the secondary antibody and Streptravidin-HRP (please inquire) together with the DAB detection system (Cat. No. 550880/7578KK). A detailed protocol of the immunohistochemical procedure is enclosed.

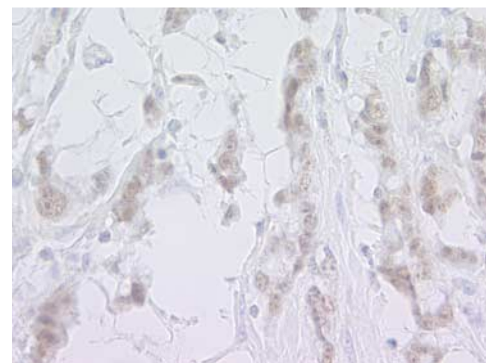

Figure: Immunohistochemistry of p53. Paraffin sections of human breast cancer tissue was reacted with the G59-12 clone. Cells expressing p53 protein can be identifed by the intense brown labeling in their cell nuclei. Magnification 20X.

OTHER APPLICATIONS

For additional applications of this clone refer to Cat. No. 554157 (Was: 14211A).

REFERENCES:

1. Vogelstein, B. 1990. Cancer. A deadly inheritance. *Nature* 348:681-2.
2. Vojtesek, B., J. Bartek, C.A. Midgley and D.P. Lane. 1992. An immunochemical analysis of the human nuclear phosphoprotein p53. New monoclonal antibodies and epitope mapping using recombinant p53. *J. Immunol. Meth.* 151:237-244.
3. Stein, L.S. G. Stoica, R. Tilley and R.C. Burghardt. 1991 Rat ovarian granulosa cell culture: a model system for the study of cell-cell communication during multistep transformation. *Cancer Res.* 51:696-706.
4. Gjerset, R.A., J. Arya, S. Volkman and M. Haas. 1992. Association of induction of a fully tumorigenic phenotype in murine radiation-induced T-lymphoma cells with loss of differentiation antigens, gain of CD44, and alterations in p53 protein levels. *Mol. Carcinog.* 5:190-198.

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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.

* Source of all serum is from the United States

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PROTOCOL FOR IMMUNOHISTOCHEMICAL STAINING OF FROZEN SECTIONS

1. Preparation of Frozen Tissues for sectioning

Materials needed:

2-methylbutane (isopentane)
Liquid nitrogen
Dry ice
Peel-Away® base molds
Frozen tissue matrix (OCT® or Cryomatrix®)
Long forceps
Necropsy tools
Superfrost Plus slides.

1. Label base mold and partially fill the mold with frozen tissue matrix.
2. Sacrifice animal by prescribed and approved euthanasia techniques.
3. Remove desired tissues and place in pre-labeled base molds filled with frozen tissue matrix. Try to arrange tissue in matrix near the bottom so tissue is easily exposed when sections are cut.
4. Plunge base mold with tissue in frozen tissue matrix into 2-methylbutane prechilled in a dewar of liquid nitrogen until the block ALMOST solidifies (30 seconds). NOTE: If the block is left in too long, it may crack.
5. Remove tissue block from 2-methylbutane and place blocked tissues on dry ice. (Tissues may be stored in the base molds or transferred to plastic bags.)
6. Store frozen tissue blocks in -70°C freezer until sectioning.
7. For sectioning, attach the frozen tissue block on the cryostat chuck. Allow tisue block to equilibrate to the cryostat temperature (-20°C) before cutting sections. Routine sections are cut at 5 microns and picked up onto slide.
8. Dry at room temperature till the sections are firmly adhered to the slide.
9. Fix sections in cold acetone (-20°C) for 2 min. Dry fixed slides completely (usually 1 hour at room temperature). Store in a -70°C freezer until use.

2. Standard Immunohistochemical Staining Procedure For Frozen Sections

Please read entire procedure before staining sections. Perform all incubations in a humid chamber and do not allow sections to dry out. Isotype and system controls should also be run and must be matched to the isotype of each primary antibody to be tested.

Materials needed:

Phosphate Buffered Saline (PBS)
Blocking buffer (please inquire)
Antibody diluent for IHC (Cat. No. 559148/70991A)
Streptravidin/ HRP (please inquire)

1. Remove frozen slides from freezer and allow to come to RT.
2. Rinse slides 2-3 times in PBS to remove frozen mounting media.
3. Apply the blocking buffer and incubate for 10 min.
4. Rinse slides in 3 changes of PBS, 2 min each.
5. Dilute primary antibody in antibody diluent for IHC (Cat. No. 559148/70991A). Apply to cover tissue sections on slide and incubate 1 hr at RT in a humid chamber.
6. Rinse slides in 3 changes of PBS, 2 min each.
7. Dilute biotinylated secondary antibody in antibody diluent (Cat. No. 559148/70991A). Apply onto tissue sections and allow to incubate at RT for 30 min.
8. Rinse slides in 3 changes of PBS, 2 min each.
9. Apply pre-diluted Strepavidin/HRP to each slide and incubate at RT 30 min.
10. Rinse slides in 3 changes of PBS, 2 min each.
11. Prepare DAB according to manufacturer's specifications. SAFETY NOTE: DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12. Drain slides and add DAB solution to the sections. Allow to incubate 5 min or less till the desired color intensity is reached.
13. Drain excess DAB on paper towel and Rinse slides well in water 3 times.
14. Counterstain slides:
    a. Dip twice in Hematoxylin.
    b. Rinse thoroughly in water.
    c. Dip twice in Bluing Reagent or dilute ammonia water.
    d. Rinse thoroughly in water.
15. Dehydrate through 4 changes of increasing grades of alcohol to 100%, clear in 3-4 changes of xylene (or xylene substitute) and coverslip.

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PROTOCOL FOR IMMUNOHISTOCHEMICAL STAINING OF PARAFFIN SECTIONS

I. Fixation and Processing of Tissue for Paraffin Sections

1. Fixation in 10% Neutral Buffered Formalin

To preserve tissue and process for paraffin embedding, cut fresh tissue into appropriate size pieces and place into 10% neutral buffered formalin.

NOTE: Tissues to be fixed and processed should be cut to a size no larger 3 mm in thickness.

Let tissue fix in the 10% formalin at RT for up to 24 hr. For small rodent tissue, we recommend fixation of 4-12 hr followed by a 9 hr processing schedule outlined below:

45 min	Station #1	10% Neutral Buffered Formalin
45 min	Station #2	50% Alcohol
45 min	Station #3	70%Alcohol
45 min	Station #4	95% Alcohol
45 min	Station #5	100% Alcohol
45 min	Station #6	100% Alcohol
45 min	Station #7	100% Alcohol
45 min	Station #8	Clearing Reagent (Xylene, substitute, etc)
45 min	Station #9	Clearing Reagent
45 min	Station #10	Clearing Reagent
1 hour	Station #11	Paraffin
1 hour	Station #12	Paraffin

Following infiltration of the tissue with paraffin, tissues can be embedded into a paraffin block for storage until microtome sectioning.

2. Fixation in Zinc Fixative

For optimal Immunohistochemical preservation of antigens, Zinc Fixative is recommended prior to processing and embedding into paraffin blocks.[1,2] Fix tissues in Zinc Fixative (please inquire) by placing freshly dissected tissue pieces (no larger than 3 mm in thickness) in Zinc Fixative and allowing to sit 24-48 hr at RT. Tissues are then to be processed according to the above processing schedule with the exception of Zinc Fixative in station #1 in place of 10% Neutral Buffered Formalin. Following infiltration of the tissue with paraffin, tissues can be embedded into a paraffin block for storage until microtome sectioning.

II. Preparation of Paraffin Sections for Immunohistochemistry

1. Section paraffin blocks at 4 microns and float on a water bath at 45^0 C containing only deionized or distilled water (no adhesives).
2. Pick up flattened sections on Superfrost PLUS® slides and dry upright overnight at RT. Store paraffin sections at RT prior to staining.
3. Deparaffinize slides in 2 changes of xylene (or xylene substitute) for 10 min each.
4. Transfer slides to 100% alcohol, 2 changes for at least 2 min each.
5. Block endogenous peroxidase activity by incubating slides for 10 min in 3% H_2O_2 in methanol.
6. Rinse slides in water and place in PBS.
7. Perform pretreatment of slides with appropriate antigen retrieval solutions if required (Please inquire with PharMingenTechnical Service Department about the different antigen retrieval systems and the protocols.
8. Rinse slides with one change PBS.
9. Apply enough of each properly diluted primary antibody to cover tissue sections on slide and incubate at 4°C overnight in a humid chamber.
10. Rinse slides in 3 changes of PBS, 2 min each. Apply proper diluted biotinylated secondary antibody and allow to incubate at RT for 30 min.
11. Rinse slides in 3 changes of PBS, 2 min each. Apply Strepavidin/HRP to each slide and incubate at RT 30 min.
12. Rinse slides in 3 changes of PBS, 2 min each.
13. Prepare DAB according to manufacturer's specifications.
    SAFETY NOTE: DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
14. Add DAB solution to each slide, making sure all the section is covered by solution. Allow to incubate 5 min or less.
15. Rinse slides well in water 3 times.
16. Counterstain slides:
    a. Dip twice in Hematoxylin.
    b. Rinse thoroughly in water.
    c. Dip twice in Bluing Reagent or dilute ammonia water.
    d. Rinse thoroughly in water.
17. Dehydrate through 4 changes of increasing grades of alcohol to 100%, clear in 3-4 changes of xylene (or xylene substitute) and coverslip.

References:

1. Nitta, H., Munger, W., Wilson, E., Ralston, R., and H. Alila; Improved In Situ Immunodetection of Leukocytes on Paraffin-Embedded Mouse Spleen. *Cell Vision.* 73,4,(no.1),1997.
2. Beckstead, Jay; A Simple Technique for Preservation of Fixation-sensitive Antigens in Paraffin-embedded Tissues. *Journal of Histochemistry and Cytochemistry.* 42,8,1994.
3. Tacha, DE, Chen T. Modified antigen retrieval procedure: calibration technique for microwave ovens. *Histotechnology* 17(4):365,1994.

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