Anti-p53 Antibody

REF : **KPIH10207**

Ver 1.0

IVD

Antibody for Qualitative Identification of p53 Antigen by light microscopy in formalin fixed, paraffin embedded (FFPE) tissue sections using immunohistochemical (IHC) detection methodology.

IVD	**For In-Vitro Diagnostic Use**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

For *In-Vitro Diagnostic Use only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN Pudgala LLP is strictly prohibited.*

REF **KPIH10207**  **3 ml / 6 ml / 0.3 ml (conc)**

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Introduction:

p53, also known as Tp53, is a DNA-binding protein which belongs to the p53 family. It contains transcription activation, DNA-binding, and oligomerization domains. p53 protein is expressed at low level in normal cells and at a high level in a variety of transformed cell lines, where it's believed to contribute to transformation and malignancy. p53 is a transcription factor whose protein levels and post-translational modification state alter in response to cellular stress. Activation of p53 begins through a number of mechanisms including phosphorylation by ATM, ATR, Chk1 and MAPKs. MDM2 is a ubiquitin ligase that binds p53 and targets p53 for proteasomal degradation. Phosphorylation, p14ARF and USP7 prevent MDM2-p53 interactions, leading to an increase in stable p53 tetramers in the cytoplasm. Tp53 regulates a large number of genes (>100 genes) that control a number of key tumor suppressing functions such as cell cycle arrest, DNA repair, senescence and apoptosis. Whilst the activation of p53 often leads to apoptosis, p53 inactivation facilitates tumor progression. It is postulated to bind to a p53-binding site and activate expression of downstream genes that inhibit growth and/or invasion, and thus function as a tumor suppressor. Mutants of p53 that frequently occur in a number of different human cancers fail to bind the consensus DNA binding site, and hence cause the loss of tumor suppressor activity. Defects in TP53 are a cause of esophageal cancer, Li-Fraumeni syndrome, lung cancer and adrenocortical carcinoma.

Intended Use:

This antibody is designed for use in qualitative identification of p53 antigen by light microscopy in formalin fixed, paraffin embedded (FFPE) tissue sections using immunohistochemical (IHC) detection methodology. Interpretation of any positive or negative staining must be complemented with the evaluation of proper known controls (positive and negative) and must be made within the context of the patient's clinical history and other diagnostic tests. Evaluation must be performed by a qualified pathologist.

Principle:

The identification of the antigen on the tissues is carried out using the above stated antibody. The antigen and antibody complex is visualized using an enzyme coupled (HRP/AP) secondary antibody with specific binding to the primary antibody, this complex is visualized by the enzymatic activation of the chromogen resulting to a visible reaction production of the antigenic site. Each and every step involves precise time and optimal temperature and the results are interpreted using a light microscope by a qualified and trained pathologist.

Materials Provided:

Rabbit Monoclonal Antibody to p53 is filtered with 0.2µm filter solution in PBS. The antibody dilution and protocol may vary depending on the specimen preparation and specific application. Optimal conditions should be determined by individual laboratory.

Handling/Storage:

Store at 2-8°C. Fresh dilutions, if required, should be prepared prior to use and are stable and steady for up to one day at room temperature (20-26°C). Diluted antibody preparations can be refrigerated or frozen for extended shelf life.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Reagent Preparation:

KRISHGEN Ready-to-Use antibodies have been optimized for use with the recommended KRISHGEN Detection System and should not require further dilution. KRISHGEN concentrated antibodies must be diluted in accordance with the recommended protocol when used with the recommended KRISHGEN Detection System

Specimen Preparation:

Staining Recommendations-
FFPE tissues are suitable for use with this primary antibody by using Krishgen Pudgala's HRP/DAB detection system.

Survival of tissue antigens for immunological staining may depend on the type and concentration of fixative, on fixation time, and on the size of the tissue specimen to be fixed. It is recommended to maintain optimal, standardized fixation conditions whenever possible in order to obtain reproducible staining results. Prolonged exposure to fixatives may result in the masking, impairment or destruction of antigens, which contribute to reduced immunostaining. The suggested tissue fixative is 10% neutral buffered formalin. Zenker's fluid, B-5 and Bouin's are alternative fixatives for the preservation of tissue antigens sensitive to routine formalin fixation.

Thickness of the sections should be 2-5um. Slides should be stained at the earliest once the sections are made as antigenicity of the cut sections may diminish over a period of time.

To ensure reliable results, it is always good to run Positive and Negative controls simultaneously with all patient specimens.

Staining Procedure:

a. **Antigen Retrieval Solution:** Use Tris-EDTA Buffer as antigen retrieval solution*.
Heat induced epitope retrieval (HIER): Retrieve sections under steam pressure for 15 minutes using buffered solutions for optimal retrieval of the epitopes. Allow the solution to cool to room temperature, Transfer the tissue sections/slides to the distilled water prior to the primary antibody application.

* Since the development of HIER, a wide range of buffered solutions have been employed. Currently, HIER solutions can be grouped in three categories based on pH and buffer compositions:
- Low pH (pH ~3-5) solutions frequently buffered by glycine-HCl.
- Low to neutral pH (pH ~ 6-7) solution buffered with citric acid.
- High pH (pH~ 8-10) buffered by Tris or EDTA.
The pH of the HIER solutions is more important than the composition of the buffer. Optimal recovery for most epitopes occurs in alkaline buffers with a pH range of 8-10. EDTA buffers are particularly effective on over-fixed specimens and for the recovery of hard to detect antigens. The high pH and EDTA based buffers are not without drawbacks. Higher pH solutions are more likely to cause loss of sections from microscope slides. In addition, EDTA solutions may result in distorted morphology as well as convoluted and bizarre shaped nuclei.

At this time, there is no "universal" HIER buffer that is optimal for all antigens. Each laboratory should evaluate the effectiveness of different HIER solutions on the recovery of the various antigens commonly evaluated in the laboratory. A common approach is the use of a buffer such as citrate for most antigens. The high pH or EDTA based solution may be reserved for those antigens which may be difficult to retrieve with citrate.

b. **Primary Antibody:** Cover the tissue sections with primary antibody and incubate for 30-60 min at room temperature.

c. **Detection System:** Refer to Krishgen Pudgala's HRP/ DAB detection system protocol for optimal staining results.

Suggestions: Refer to the KRISHGEN website for guidance on specific staining protocol or other requirements.

Expected Results:

This antibody stains membrane in positive cells in formalin fixed, paraffin embedded tissue sections (Fig.1). An example image of a tissue section stained with this antibody can be found on the product page on the KRISHGEN website. Interpretation of the staining result is solely the responsibility of the user.

A qualified experienced/trained pathologist must interpret the results in the patient's sample along with the positive and negative controls.

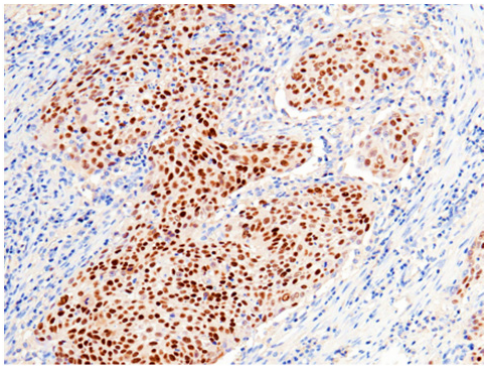

Fig.1. Immunochemical staining of human p53 in human tonsil with Rabbit monoclonal antibody, formalin-fixed paraffin embedded sections.

Limitations of Method:

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis. Improper tissue handling and processing prior to immune-staining can lead to inconsistent results. Variations in embedding and fixation or the nature of the tissue may lead to variations in results.

Endogenous peroxidase activity or pseudo peroxidase activity in erythrocytes and tissue biotin may result in non-specific staining based on the detection system employed. Tissues containing Hepatitis B Surface Antigen (HBsAg) may give false positive with horseradish peroxidase systems. Improper counterstaining and mounting may compromise the interpretation of results.

Quality Control:
A positive and negative tissue control must be run with every staining procedure performed for monitoring the right performance of processed tissue and test reagents. A negative tissue controls offer an indication of non-specific background staining. If the results are not expected in positive and negative controls the test must be considered invalid and entire procedure must be cross verified. Individual laboratory must establish their own quality control to validate the process and antibody when opened a vial. Refer to KRISHGEN detection system documents for guidance on general quality control procedures.

Performance characteristics:

KRISHGEN products will undergo through a quality control check before it is released to the market. The antibody showed consistent specific and sensitive staining on the multiple positive tissue controls tested, by inter run, intra run and lot based studies.

Safety Precautions:

- **This kit is For In-vitro Diagnostic Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.

- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

Troubleshooting:

- Follow the recommended protocol provided in the data sheet.
- Do not allow the section to dry out during the entire IHC process
- Extreme or incomplete counterstaining may compromise the interpretation of the results
- If unusual results occur, contact KRISHGEN PUDGALA LLP's Technical Support at +91-22-49198700.

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THANK YOU FOR USING KRISHGEN PRODUCT!

Krishgen Pudgala LLP

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CE Marked	Europe
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