

**WT-1 (Clone: GUDP)
Rabbit Monoclonal Antibody**

PRODUCT INFORMATION:
MR1296 6ml Ready to use
MR1296 3ml Ready to use
MRC1296 1ml Concentrated
MRC1296 0.5ml Concentrated
MRC1296 0.1ml Concentrated
MRH1296 6ml Ready to use
MRH1296 3ml Ready to use

PERFORMANCE CHARACTERISTICS:
Localization: Nucleus
Retrieval Buffer: Tris-EDTA, pH 9.0
Incubation: 60 minutes
Positive control: Tonsil, Thymus

INTENDED USE

For research use only
 This antibody is intended for use in qualitatively identify WT-1 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. A qualified and trained pathologist must perform evaluation of the test. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains

SUMMARY AND EXPLANATION

The WT1 gene located at chromosome 11p13 codes for a transcription factor, a DNA-binding nucleoprotein that plays a role primarily in the development of genitourinary organs. There are at least eight isoforms ranging between 52 and 62 kDa produced by a combination of alternative splicing and RNA editing. WT1 is synthesized and reside in the cytoplasm in an inactive form. When activated through phosphorylation it is translocated to the nucleus. WT1 influences cell proliferation by suppressing bcl-2 and regulating cadherin and p53. In normal epithelia, nuclear WT1 expression is largely restricted to ovary (surface epithelium and inclusion cysts) and fallopian tube, while WT1 is not found in endometrial or cervical epithelium. As regards nonepithelial cells, nuclear WT1 is found in mesothelium and some submesothelial stromal cells, stromal cells of the female genital tract, testicular non-germinal cells, and kidney (podocytes). In tumor tissues, WT1 is detected in tumor cells of Wilms' Tumor (also known as nephroblastoma) and mesothelioma. Additionally, WT1 expression has been found in ovarian serous carcinomas and some breast carcinomas. WT1 is particularly used for distinguishing malignant mesothelioma and ovarian serous carcinoma from non-serous carcinomas. As for malignant mesothelioma, calretinin and WT1 are superior to cytokeratin 5/6, N-cadherin and thrombomodulin. WT1 is also applicable for the differential diagnostic of small cell childhood tumors.

PRINCIPLE OF THE PROCEDURE

The identification of the antigen on the FFPE 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.

REAGENT PROVIDED

Concentrated format: Antibody to WT-1 is affinity purified and diluted in antibody diluent with 1% bovine serum albumin (BSA) and 0.05% of sodium azide (NaN₃).
Recommended dilutions: 1:50-1:100
 The antibody dilution and protocol may vary depending on the specimen preparation and specific application. Optimal conditions should be determined by individual laboratory.
Pre-diluted format: PathnSitu's ready to use antibodies are pre-titrated to optimal staining conditions. Further dilution will affect the efficacy of the antibody and may yield to sub-optimal staining.
Immunogen: Synthetic peptide corresponding to WT-1 residues within aa1-100 of WT-1 was used as immunogen
Host, Isotype: Rabbit, IgG

STORAGE AND HANDLING

Storage Recommendations: Store at 2-8°C. When stored at the appropriate conditions, the antibody is stable until expiry. Do not use the antibody after expiration date provided on the vial in any condition.
 To ensure proper reagent delivery and stability, replace the dispenser cap after every use and immediately place the vial into the refrigerated conditions in an upright position. The contents of the vial should be used within 9 months from the opening of the vial.

SPECIMEN PREPARATION

Staining Recommendations:
 Routinely processed, FFPE tissues are suitable for use with this primary antibody, when used PathnSitu's Poly Excel HRP/DAB detection system. The recommended tissue fixative is 10% neutral buffered formalin. Variable results may occur as a result of prolonged fixation or special processes such as decalcification. Thickness of the sections should be 2-5µm. Slides should be stained once the sections are made as antigenicity of the cut sections may diminish over a period of time. It is recommended to stain known positive and negative controls simultaneously with unknown specimens.

PRECAUTIONS

1. This product should be used by qualified and trained professional users only
2. The product contains < 0.1% of sodium azide as preservative and is not classified hazardous, refer MSDS for further details
3. As with any product derived from biological sources, proper handling procedures should be used
4. Do not use reagents after expiration date
5. Use protective clothing and gloves, while handling reagents
6. All hazardous materials should be disposed according to local state and federal regulations
7. Avoid microbial contamination of reagents as it may lead to incorrect results

STAINING PROCEDURE

Antigen Retrieval Solution: Use **Tris-EDTA Buffer (Cat#PS009)** as antigen retrieval solution.
Heat Retrieval Method: Retrieve sections under steam pressure for 20 minutes using PathnSitu's MERS (Multi Epitope Retrieval System) for optimal retrieval of the epitopes, allow solution to cool at the room temperature, transfer the tissue sections/slides to the distilled water prior to the primary antibody application.
Primary Antibody: Cover the tissue sections with primary antibody and incubate for 60 min at room temperature when used PathnSitu's PolyExcel Detection System.
Detection System: Refer to PathnSitu's PolyExcel HRP/ DAB detection system protocol for optimal staining results.

QUALITY CONTROL

The recommended positive tissue control for WT-1 is Tonsil, Thymus. A positive and negative tissue control must be run with every staining procedure performed for monitoring the correct performance of processed tissue and test reagents. A negative tissue controls provide 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.

INTERPRETATION OF RESULTS

WT-1 stains the Nucleus. A qualified experienced/trained pathologist must interpret the results in the patient's sample along with the positive and negative controls.

PERFORMANCE CHARACTERISTICS

PathnSitu products will undergo a thorough 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. The antibody is stable for the expiry mentioned on the labels which is determined by real time or accelerated methods.

TROUBLESHOOTING

1. Follow the antibody specific protocol recommendations according to data sheet provided
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, tissue processing, antibody freezing and thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping or inaccurate results
3. Do not allow the section to dry out during the entire IHC process
4. Excessive or incomplete counterstaining may compromise the interpretation of the results
5. If unusual results occur, contact PathnSitu's Technical Support at +91-40-2701 5544 or E-mail: techsupport@pathnsitu.com

LIMITATIONS AND WARRANTY

Authorized and skilled/trained personnel only may use the product. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic test results. A qualified trained pathologist must perform the evaluation of the test results. There are no warranties, expressed or implied, which extend beyond the description. PathnSitu is not liable for property damage, personal injury, time or effort on economic loss caused by this product.

BIBLIOGRAPHY

1. Carpentieri DF et al. Mod Pathol. 2002 Oct; 15(10):1080-6.
2. Goldstein NS et al. Am J Clin Pathol. 2002 Apr; 117(4):541-5.

EXPLANATION OF SYMBOLS

LOT- Lot number / Batch number



RUO Research use only



Storage limitation