# WT1 [rWT1/857]

Concentrated and Prediluted Monoclonal Antibody
901-3238-060123

BIOCARE MEDICAL

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|---|---|---|
| **Catalog Number:** | **ACI 3238 A, C** | **API 3238 AA** |
| **Description:** | 0.1, 1.0 mL, conc. | 6.0 mL, RTU |
| **Dilution:** | 1:100 | Ready-to-use |
| **Diluent:** | Renoir Red | N/A |

**Intended Use:**
For In Vitro Diagnostic Use
WT1 [rWT1/857] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of WT1 protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

**Summary and Explanation:**
WT1 is a gene involved in the induction of Wilms' tumor. In normal human tissues, WT1 mRNA has been observed in kidney, spleen and gonadal ridge mesoderm (1). WT1 expression has also been observed in sertoli cells of testes and in granulosa cells of the ovary. In tumors, WT1 has been demonstrated in Wilms' tumors and in the majority of mesotheliomas (nuclear and paranuclear staining) (2). WT1 has also been demonstrated in the majority of acute leukemias, but not in cells from chronic myelogenous leukemia (3). Cytoplasmic staining has been observed in some cases of adenocarcinoma and may represent cross-reactivity with an epitope unrelated to WT1 (2).

**Principle of Procedure:**
Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal
**Species Reactivity:** Human; others not tested
**Clone:** rWT1/857
**Isotype:** IgG1/kappa
**Protein Concentration:** Call for lot specific Ig concentration.
**Epitope/Antigen:** Recombinant full-length human WT1 protein
**Cellular Localization:** Nuclear and cytoplasmic
**Positive Tissue Control:** Wilms' tumor, mesothelioma, or normal kidney

**Known Applications:**
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
**Supplied As:** Buffer with protein carrier and preservative

**Storage and Stability:**
Store at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

**Protocol Recommendations (intelliPATH FLX® and manual use):**
**Peroxide Block:** Block for 5 minutes with Peroxidazed 1.
**Pretreatment:** Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

**Protocol Recommendations (intelliPATH FLX and manual use) Cont'd:**
**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Background Punisher.
**Primary Antibody:** Incubate for 30 minutes at RT.
**Probe:** Incubate for 10 minutes at RT with a secondary probe.
**Polymer:** Incubate for 10-20 minutes at RT with a tertiary polymer.
**Chromogen:** Incubate for 5 minutes at RT with Biocare's DAB - OR - Incubate for 5-7 minutes at RT with Warp Red.
**Counterstain:**
Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.
**Technical Note:**
This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

**Performance Characteristics:**
Sensitivity, specificity and cross-reactivity are summarized in Tables 1 and 2, respectively.

**Limitations:**
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

**Quality Control:**
Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

**Precautions:**
1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide ($NaN_3$) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (4)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (5)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at http://biocare.net.

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**Troubleshooting:**
Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

**References:**
1. Wilm B, Muñoz-Chapuli R. The Role of WT1 in Embryonic Development and Normal Organ Homeostasis. Methods Mol Biol. 2016;1467:23-39.
2. Ordonez NG. The immunohistochemical diagnosis of mesothelioma: a comparative study of epithelioid mesothelioma and lung adenocarcinoma. Am J Surg Pathol. 2003 Aug;27(8):1031-51.
3. Rosenfeld C, Cheever MA, Gaiger A. WT1 in acute leukemia, chronic myelogenous leukemia and myelodysplastic syndrome: therapeutic potential of WT1 targeted therapies. Leukemia. 2003 Jul;17(7):1301-12.
4. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
5. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

**Table 1:** Sensitivity and specificity were determined by testing formalin-fixed, paraffin-embedded diseased tissues.

| Tissue | Positive Cases | Total Cases |
|---|---|---|
| Wilms' Tumor | 8 | 8 |
| Mesothelioma | 20 | 20 |
| Bladder Cancer | 40 | 40 |
| Breast Cancer | 23 | 23 |
| Colon Cancer | 14 | 19 |
| Lung Cancer | 18 | 20 |
| Ovarian Cancer | 37 | 40 |
| Prostate Cancer | 14 | 15 |
| Renal Cancer | 29 | 40 |
| Melanoma | 3 | 3 |

**Table 2:** Tissue cross-reactivity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

| Tissue | Positive Cases | Total Cases |
|---|---|---|
| Cerebrum | 2 | 2 |
| Cerebellum | 3 | 3 |
| Adrenal | 3 | 3 |
| Ovary | 10 | 11 |
| Pancreas | 3 | 3 |
| Parathyroid | 3 | 3 |
| Pituitary | 2 | 3 |
| Testis | 3 | 3 |
| Thyroid | 3 | 3 |
| Breast | 2 | 3 |
| Spleen | 3 | 3 |
| Tonsil | 4 | 4 |
| Thymus | 3 | 3 |
| Lung | 8 | 9 |
| Heart | 3 | 3 |
| Esophagus | 3 | 3 |
| Stomach | 3 | 3 |
| Small Intestine | 3 | 3 |
| Colon | 7 | 7 |
| Liver | 3 | 3 |
| Salivary Gland | 3 | 3 |
| Kidney | 12 | 12 |
| Bladder | 5 | 7 |
| Prostate | 4 | 5 |
| Uterus | 3 | 3 |
| Cervix | 2 | 3 |
| Skeletal Muscle | 3 | 3 |
| Skin | 3 | 3 |
| Peripheral Nerve | 3 | 3 |
| Linging Cells | 1 | 1 |
| Lymph Node | 1 | 1 |

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