

TIGIT (RM) [BLR047F]

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-3243-060123

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M E D I C A L

Catalog Number:	ACI 3243 A, C	API 3243 AA	VLTR 3243 G20
Description:	0.1, 1.0 mL, conc.	6.0 mL, RTU	20 mL, RTU
Dilution:	1:100	Ready-to-use	Ready-to-use
Diluent:	Da Vinci Green	N/A	N/A

Intended Use:

For In Vitro Diagnostic Use

TIGIT (RM) [BLR047F] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of TIGIT 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:

T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory domain (TIGIT) is a transmembrane glycoprotein receptor expressed in regulatory and memory T cells, natural killer (NK), and activated T cells (1). As a member of the immunoglobulin superfamily, TIGIT has co-inhibitory effects on T-cell dependent immune responses, playing an important role in transplantation tolerance and tumor immune surveillance (2,3). Several studies indicate that TIGIT exhibits synergistic function with the PD-1/PD-L1 pathway in the inhibition of T-cell proliferation. Co-blockade of TIGIT in conjunction with other checkpoint receptors, such as PD-1, has been investigated as a promising immunotherapy in multiple ongoing clinical trials (4).

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-step or two-step detection procedure can be applied. A one-step procedure will feature an enzyme labeled polymer that binds the primary antibody. A two-step procedure will feature a linker antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Rabbit monoclonal

Species Reactivity: Human; others not tested

Clone: BLR047F

Isotype: IgG

Protein Concentration: Call for lot specific Ig concentration.

Epitope/Antigen: TIGIT

Cellular Localization: Cytoplasmic

Positive Tissue Control: Tonsil

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative

Storage and Stability:

Store at 2°C to 8°C. 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 2°C to 8°C.

Protocol Recommendations (VALENT® Automated Slide Staining Platform):

VLTR3243 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:

Deparaffinization: Deparaffinize for 8 minutes with Val DePar.

Pretreatment: Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X (use at 1X).

Protocol Recommendations (VALENT Automated Slide Staining Platform) Cont'd:

Enzyme: Incubate for 10 minutes with Val Zyme Pronase (1:25 mix)

Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.

Protein Block (Optional): Incubate for 10-20 minutes at RT with Val Background Block.

Primary Antibody: Incubate for 30 minutes.

Secondary: N/A

Linker: Incubate for 10 minutes with Val Universal Linker.

Polymer: Incubate for 20 minutes with Val Universal Polymer.

Chromogen: Incubate for 5 minutes with Val DAB.

Counterstain: Counterstain for 5 minutes with Val Hematoxylin.

Protocol Recommendations (intelliPATH FLX® and manual use):

Peroxide Block: Block for 5 minutes with Peroxidized 1.

Pretreatment: Perform heat retrieval using Borg Decloaker. Refer to the Borg Decloaker data sheet for specific instructions.

Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.

Primary Antibody: Incubate for 30 minutes at RT.

Probe: N/A

Polymer: Incubate for 30 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 (NaN₃) 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

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Precautions Cont'd:

Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (5)

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. (6)

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>.

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. Kurtulus S, *et al.* TIGIT predominantly regulates the immune response via regulatory T cells. *J Clin Invest.* 2015;125:4053–4062.

2. Johnston RJ, *et al.* The immunoreceptor TIGIT regulates antitumor and antiviral CD8(+) T cell effector function. *Cancer Cell.* 2014;26:923–937.

3. Zhang Q, *et al.* Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity. *Nat Immunol.* 2018;19:723–732.

4. Qin S, *et al.* Novel immune checkpoint targets: moving beyond PD-1 and CTLA-4. *Mol Cancer.* 2019;18(1):155. 2019 Nov 6.

5. 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."

6. 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
Breast Cancer	14	23
Colon Cancer	25	37
Lung Adenocarcinoma	19	23
Lung Squamous Cell Carcinoma	10	19
Prostate Cancer	16	39
Melanoma	29	39
Ovarian Cancer	18	40

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

Tissue	Positive Cases	Total Cases
Cerebrum	3	3
Cerebellum	3	3
Adrenal	2	3
Ovary	0	11
Pancreas	2	3
Parathyroid	0	3
Pituitary	1	2
Testis	0	3
Thyroid	2	3
Breast	0	3
Spleen	3	3
Tonsil	10	10
Thymus	3	3
Bone Marrow	0	3
Lung	0	3
Heart	0	3
Esophagus	0	1
Stomach	0	3
Small Intestine	0	3
Colon	0	11
Liver	0	3
Salivary Gland	0	3
Kidney	0	3
Prostate	0	11
Uterus	0	3
Cervix	0	1
Skeletal Muscle	0	3
Skin	0	3
Peripheral Nerve	0	1
Lining Cells	0	3