

TIGIT [BC41]

Concentrated and Prediluted Monoclonal Antibody
902-3254-021221

BIOCARE
M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACR 3254 A, C	0.1, 1.0 mL	1:100	Da Vinci Green
Predilute	APR 3254 AA	6.0 mL	Ready-to-use	N/A
UltraLine – For BenchMark	AVR 3254 G	6.0 mL	Ready-to-use	N/A

Intended Use:

For Research Use Only. Not for use in diagnostic procedures.

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-, 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: BC41

Isotype: IgG1/kappa

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.

Staining Protocol Recommendations (intelliPATH FLX® and manual use):

Peroxide Block: Block for 5 minutes with Peroxidase 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.

Staining Protocol Recommendations (intelliPATH FLX and manual use) Cont'd:

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 2 detection system. Use TBS for washing steps.

Staining Protocol Recommendations (Ventana BenchMark ULTRA):

AVR3254 is intended for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

Template/Detection: OptiView DAB IHC

Pretreatment Protocol: CC1 32 minutes

Peroxidase: Pre Primary Peroxidase Inhibitor

Primary Antibody: 16 minutes, 36°C

Amplification Kit: 4 minutes, 4 minutes

Performance Characteristics:

Sensitivity, specificity and cross-reactivity are summarized in Tables 1 and 2, respectively.

Limitations:

This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

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 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>.

Technical Support:

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.



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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.

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

Tissue	Positive Cases	Total Cases
Breast Cancer (IDC)	9	24
Colon Cancer	0	40
Lung Adenocarcinoma	1	24
Lung Squamous Cell Carcinoma	0	21
Prostate Cancer	3	39
Melanoma	12	40
Ovarian Cancer	10	40

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

Tissue	Positive Cases	Total Cases
Cerebrum	0	2
Cerebellum	0	2
Adrenal	2	2
Ovary	0	11
Pancreas	2	3
Parathyroid	3	3
Pituitary	1	1
Testis	0	3
Thyroid	0	3
Breast	0	2
Spleen	3	3
Tonsil	9	9
Thymus	0	3
Bone Marrow	0	2
Lung	0	3
Heart	0	3
Esophagus	0	3
Stomach	0	3
Small Intestine	0	3
Colon	0	10
Liver	2	2
Salivary Gland	2	2
Kidney	0	3
Prostate	0	10
Uterus	0	2
Cervix	2	2
Skeletal Muscle	0	1
Skin	1	10
Peripheral Nerve	0	2
Lining Cells	1	2