

TIM3 [BLR033F]

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-3258-031221

BIOCARE
M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACI 3258 A, C	0.1, 1.0 mL	1:50	Renoir Red
Predilute	API 3258 AA	6.0 mL	Ready-to-use	N/A

Intended Use:

For In Vitro Diagnostic Use

TIM3 [BLR033F] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of TIM3 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:

TIM3 or T-cell Immunoglobulin domain and Mucin domain 3 can be expressed on both tumor and immune cells including T helper type 1 (Th1) cells, Th17 and CD8+ T cells, tumor infiltrating lymphocytes (TILs), regulatory T cells and innate immune cells (1). TIM3 acts as a negative regulator of Th1/Tc1 (T cytotoxic type 1) function by triggering cell death upon interaction with its ligand, galectin-9 (2,3).

Co-blockade of TIM3 and programmed cell death 1 (PD1) can result in tumor regression in preclinical models of melanoma, colorectal cancer, and acute myeloid leukemia (AML). In patients with more advanced cancers, co-blockade of TIM3 and PD1 can improve anticancer T cell response (4). An increasing number of preclinical studies have reported that TIM3 may improve immunotherapy outcomes in various cancers including melanoma, ovarian cancer, colon cancer, AML, and gastric cancer (5).

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: BLR033F

Isotype: IgG

Protein Concentration: Call for lot specific Ig concentration.

Epitope/Antigen: TIM3

Cellular Localization: Membrane

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 (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 Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (6)

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. (7)

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Precautions Cont'd:

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. Friedlaender A, et al. New emerging targets in cancer immunotherapy: the role of TIM3. *ESMO Open*. 2019;4(Suppl 3): e000497.
2. Zhu C, et al. TIM-3 and its regulatory role in immune responses. *Curr Top Microbiol Immunol*. 2010 Aug, 350: 1–15.
3. Syn, et al. De-novo and acquired resistance to immune checkpoint targeting. *The Lancet Oncology* 2017; 18 (12): e731–e41.
4. Wolf Y, et al. TIM3 comes of age as an inhibitory receptor. *Nat Rev Immunol*. 2020;20(3):173-85.
5. He Y, et al. TIM-3, a promising target for cancer immunotherapy. *Onco Targets Ther*. 2018; 11:7005-09.
6. 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."
7. 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 (IDC)	7	24
Colon Adenocarcinoma	14	40
Lung Adenocarcinoma	19	24
Lung Squamous Cell Carcinoma	11	22
Prostate Adenocarcinoma	6	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	2
Pancreas	0	3
Parathyroid	0	3
Pituitary	2	2
Testis	0	2
Thyroid	0	2
Breast	1	3
Spleen	2	2
Tonsil	3	3
Thymus	0	2
Bone Marrow	2	2
Lung	2	2
Heart	0	3
Esophagus	2	3
Stomach	1	3
Small Intestine	2	2
Colon	3	3
Liver	2	2
Salivary Gland	1	3
Kidney	3	3
Prostate	0	3
Uterus	2	2
Cervix	0	2
Skeletal Muscle	1	2
Skin	1	1
Peripheral Nerve	0	1
Lining Cells	0	2