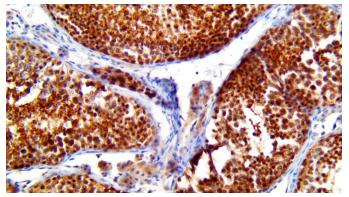


TIGIT

Clone: BSB-152 Mouse Monoclonal





Inset: IHC of TIGIT on a FFPE Testis Tissue

Intended Use

For In Vitro Diagnostic Use.

This antibody is intended for use in Immunohistochemical applications on formalin-fixed paraffin-embedded tissues (FFPE), frozen tissue sections, and cell preparations. Interpretation of results should be performed by a qualified medical professional.

Immunogen

Recombinant human TIGIT protein.

Summary and Explanation

TIGIT (T cell immunoreceptor with Ig and ITIM domains) is an immune receptor present on some T cells and Natural Killer Cells (NK). It is also identified as WUCAM and Vstm3. TIGIT could bind to CD155 (PVR) on dendritic cells (DCs), macrophages, and other immune cells with high affinity, and also to CD112 (PVRL2) with lower affinity. Research has shown that TIGIT-Fc fusion protein could interact with PVR on dendritic cells and increase its IL-10 secretion level/decrease its IL-12 secretion level under LPS stimulation, and also inhibit T cell activation *in vivo*. TIGIT's inhibition of NK cytotoxicity can be blocked by antibodies against its interaction with PVR, and the activity is directed through its ITIM domain.

TIGIT is expressed on regulatory T cells (Tregs) and on activated CD4+ T, CD8+ T, and NK cells. TIGIT and PD-1 has been shown to be over-expressed on tumor antigen-specific (TA-specific) CD8+ T cells and CD8+ tumor infiltrating lymphocytes (TILs) from individuals with melanoma. Blockade of TIGIT and PD-1 led to increased cell proliferation, cytokine production, and degranulation of TA-specific CD8+ T cells and TIL CD8+ T cells, therefore it can be considered an immune checkpoint. Co-blockade of TIGIT and PD-1 pathways elicits tumor rejection in preclinical murine models.

Antibody Type	Mouse Monoclonal	Clone	BSB-152	
Isotype	lgG2b	Reactivity	Paraffin, Frozen	
Localization	Cytoplasmic, Membranous	Species Reactivity	Human, Rat	
Control	Cervix, Fallopian Tube, Skin, Prostate, Testis, Transitional Cell Carcinoma, Papillary Thyroid Carcinoma, Lung Adenocarcinoma			
Application	Immunotherapy, Melanoma and Skin Cancer			

Presentation

Anti-TIGIT is a Mouse Monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

Catalog No.	Presentation	Dilution	Volume	
BSB-3751-3	Predilute	Ready-to-Use	3.0 mL	
BSB-3751-7	Predilute	Ready-to-Use	7.0 mL	
BSB-3751-15	Predilute	Ready-to-Use	15.0 mL	
BSB-3751-01	Concentrate	1:50-1:200	0.1 mL	
BSB-3751-05	Concentrate	1:50-1:200	0.5 mL	
BSB-3751-1	Concentrate	1:50-1:200	1.0 mL	

Control Slides Available

Catalog No.	Quantity		
BSB-3751-CS	5 slides		

Storage Store at 2-8°C (Control Slides: Store at 20-25°C)

Precautions

- 1. For professional users only. Results should be interpreted by a qualified medical professional.
- 2. This product contains <0.1% sodium azide (NaN $_3$) as a preservative. Ensure proper handling procedures are used with this reagent.
- 3. Always wear personal protective equipment such as a laboratory coat, goggles, and gloves when handling reagents.
- 4. Dispose of unused solution with copious amounts of water.
- 5. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
- 6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
- 7. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
- 8. For additional safety information refer to Safety Data Sheet for this product.
- 9. For complete recommendations for handling biological specimens, please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories" (see References in this document).

Stability

This product is stable up to the expiration date on the product label.

Do not use after expiration date listed on the package label. Temperature fluctuations should be avoided. Store appropriately when not in use, and avoid prolonged exposure to room temperature conditions.

Specimen Preparation

Paraffin sections: The antibody can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation for best results. Pre-treatment of tissues with heat-induced epitope retrieval (HIER) is recommended using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023), ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033), or ImmunoDNA Digestor (BSB 0108-0112). See reverse side for complete protocol. Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

Frozen sections and cell preparations: The antibody can be used on acetone-fixed frozen sections and acetone-fixed cell preparations.

IHC Protocol

- 1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positively charged slides such as Bio SB Hydrophilic Plus Slides (BSB 7028). 2. Air dry for 2 hours at 58° C.
- 3. Deparaffinize, dehydrate and rehydrate tissues.
- 4. Subject tissues to heat induced epitope retrieval (HIER) using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033).
- 5. Any of three heating methods may be used:

a. TintoRetriever Pressure Cooker or Equivalent

Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA and place on trivet in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 15 minutes. Open and immediately transfer slides to room temperature.

b. TintoRetriever PT Module or Water Bath Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA at 95°-99° C. Incubate for 30-60 minutes

c. Conventional Steamer Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a steamer, cover and steam for 30-60 minutes.

- 6. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
- 7. For manual IHC, perform antibody incubation at ambient temperature. For automated IHC methods, perform antibody incubation according to instrument manufacturer's instructions.
- 8. Wash slides with ImmunoDNA washer or DI water.
- 9. Continue IHC protocol. Wash slides between each step with ImmunoDNA washer solution.

Abbreviated Immunohistochemical Protocol

Step	ImmunoDetector AP/HRP	PolyDetector AP/HRP	PolyDetector Plus HRP	
Peroxidase/AP Blocker	5 min.	5 min.	5 min	
Primary Antibody	30-60 min.	30-60 min.	30-60 min.	
1st Step Detection	10 min.	30-45 min.	15 min.	
2nd Step Detection	10 min.	Not Applicable	15 min.	
Substrate- Chromogen	5-10 min.	5-10 min.	5-10 min.	
Counterstain / Coverslip	Varies	Varies	Varies	

Mounting Protocols

For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent based resin such as PermaMounter (BSB 0094-0097), refer to PI0174 or PI0097.

Product Limitations

Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized, and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a qualified medical professional.

References

- 1. Yu X, Harden K, Gonzalez LC, et al. The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. Nat Immunol. 2009;10(1):48-57. doi:10.1038/ni.1674
- 2. Levin SD, Taft DW, Brandt CS, et al. Vstm3 is a member of the CD28 family and an important modulator of T-cell function. Eur J Immunol. 2011;41(4):902-915. doi:10.1002/eji.201041136
- 3. Chauvin JM, Pagliano O, Fourcade J, et al. TIGIT and PD-1 impair tumor antigen-specific CD8⁺ T cells in melanoma patients. J Clin Invest. 2015;125(5):2046-2058. doi:10.1172/JCI80445
- 4. Johnston RJ, Comps-Agrar L, Hackney J, et al. The immunoreceptor TIGIT regulates antitumor and antiviral CD8(+) T cell effector function. Cancer Cell. 2014;26(6):923-937. doi:10.1016/j.ccell.2014.10.018 5. Argast G, Cancilla B, Cattaruzza F, et al. Abstract 5627: Anti-TIGIT biomarker study: Inhibition of TIGIT induces loss of Tregs from tumors and requires effector function for tumor growth inhibition. Cancer Research. 2018;78:5627-5627. doi:10.1158/1538-7445.AM2018-5627 6. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe WorkPractices in Human and Animal Medical Diagnostic Laboratories. Supplement / Vol. 61, January 6, 2012. https://www.cdc.gov/mmwr/pdf/other/su6101.pdf

Symbol Key / Légende des symboles/Erläuterung der Symbole

EC RE	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands	1	Storage Temperature Limites de température Zulässiger Temperaturbereich	***	Manufacturer Fabricant Hersteller	REF	Catalog Number Référence du catalogue Bestellnummer
IVD	In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum	(i	Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	\searrow	Expiration Date Utiliser jusque Verwendbar bis	LOT	Lot Number Code du lot Chargenbezeichnung



