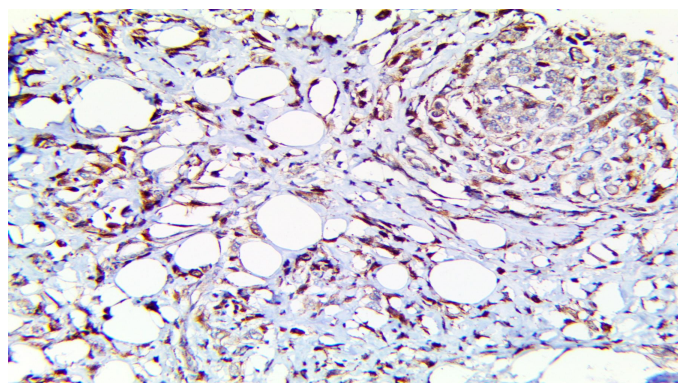


TIM-3/HAVCR2/CD366

Clone: BSB-163
Mouse Monoclonal



Inset: IHC of TIM-3/HAVCR2/CD366 on a FFPE Ductal Breast Carcinoma 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 extracellular domain of the human TIM3 protein.

Summary and Explanation

T-cell Immunoglobulin and Mucin-domain Containing-3 (TIM-3), also known as Hepatitis A virus cellular receptor 2 (HAVCR2), is a protein that in humans is encoded by the *HAVCR2* gene. TIM-3 is an immune checkpoint and together with other inhibitory receptors including programmed cell death protein 1 (PD-1) and lymphocyte activation gene 3 protein (LAG3) mediate CD8⁺ T-cell exhaustion. TIM-3 expression is up-regulated in tumor-infiltrating lymphocytes in Lung, Gastric, Head & Neck Cancers, Schwannoma, Melanoma and Follicular B-cell Non-Hodgkin Lymphoma. The TIM-3 pathway may interact with the PD-1 pathway in the dysfunctional CD8⁺ T cells and Tregs in cancer. TIM-3 is mainly expressed on activated CD8⁺ T cells and suppresses macrophage activation following PD-1 inhibition.

Upregulation has been observed in tumors progressing after anti-PD-1 therapy. It has been reported that early breast cancer patients with TIM-3⁺ iTILs have significantly improved breast cancer-specific survival whereas TIM-3⁺ sTILs did not reach statistical significance and it was concluded that the presence of TIM-3⁺ iTILs is an independent favorable prognostic factor in the whole cohort as well as among ER negative patients. In myelogenous leukemia (AML), upregulated TIM-3 during AML could reduce cytokine production. Co-expression of PD-1 and TIM-3 was correlated with AML progression. In follicular B-cell non-Hodgkin lymphoma, TIM-3 was expressed on nearly 35% of lymph node CD4⁺ and CD8⁺ T cells and could mediate T-cells exhaustion. In glioma patients, TIM-3 was correlated with cancer immune escape and might be a potent target. In colorectal cancer, upregulation of TIM-3 could restrict T-cell

responses and might participate in tumorigenesis. The expression of TIM-3 might be an independent prognostic factor for colorectal cancer.

Antibody Type	Mouse Monoclonal	Clone	BSB-163
Isotype	IgG2c	Reactivity	Paraffin, Frozen
Localization	Cytoplasmic	Species Reactivity	Human
Control	Colon, Testis, Tonsil, Liver		
Application	Immunotherapy, Leukemia and Histiocytic Cancer, Lymphoma, Breast Cancer, Melanoma and Skin Cancer		

Presentation

Anti-TIM-3/HAVCR2/CD366 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.

<i>Catalog No.</i>	<i>Presentation</i>	<i>Dilution</i>	<i>Volume</i>
BSB-3752-3	Predilute	Ready-to-Use	3.0 mL
BSB-3752-7	Predilute	Ready-to-Use	7.0 mL
BSB-3752-15	Predilute	Ready-to-Use	15.0 mL
BSB-3752-01	Concentrate	1:25-1:100	0.1 mL
BSB-3752-05	Concentrate	1:25-1:100	0.5 mL
BSB-3752-1	Concentrate	1:25-1:100	1.0 mL

Control Slides Available

<i>Catalog No.</i>	<i>Quantity</i>
BSB-3752-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 (NaN₃) 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. Entrez Gene Summary for HAVCR2 Gene. <https://www.genecards.org/cgi-bin/carddisp.pl?gene=HAVCR2>.
2. Blackburn SD, Shin H, Haining WN, et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. Nat Immunol. 2009;10(1):29-37. doi:10.1038/ni.1679
3. Gao X, Zhu Y, Li G, et al. TIM-3 expression characterizes regulatory T cells in tumor tissues and is associated with lung cancer progression. PLoS One. 2012;7(2):e30676. doi:10.1371/journal.pone.0030676
4. Shayan G, Srivastava R, Li J, Schmitt N, Kane LP, Ferris RL. Adaptive resistance to anti-PD1 therapy by Tim-3 upregulation is mediated by the PI3K-Akt pathway in head and neck cancer. Oncoimmunology. 2016;6(1):e1261779. Published 2016 Dec 23. doi:10.1080/2162402X.2016.1261779
5. Burugu S, Gao D, Leung S, Chia SK, Nielsen TO. TIM-3 expression in breast cancer. Oncoimmunology. 2018;7(11):e1502128. Published 2018 Aug 23. doi:10.1080/2162402X.2018.1502128
6. He Y, Cao J, Zhao C, Li X, Zhou C, Hirsch FR. TIM-3, a promising target for cancer immunotherapy. Onco Targets Ther. 2018;11:7005-7009. Published 2018 Oct 16. doi:10.2147/OTT.S170385
7. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe Work Practices 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

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