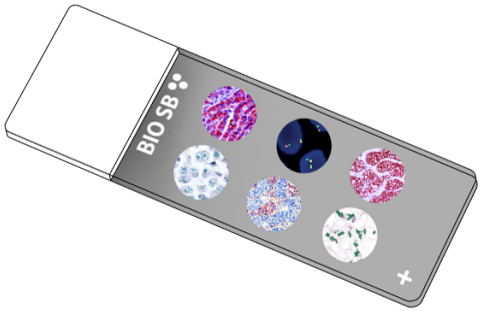
# TIM-3/HAVCR2/CD366 Control Slides

CE IVD




**Intended Use**
For In Vitro Diagnostic Use.

**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 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.

**Presentation**
Five slides of TIM-3/HAVCR2/CD366 positive tissues, each mounted on Hydrophilic Plus Slides, provided in a plastic mailer.

| *Catalog No.* | *Quantity* |
|---|---|
| BSB-9411-CS | 5 slides |
| BSB-3752-CS | 5 slides |

**Storage** Store at 20-25°C

**Precautions**
1. For professional users only. Results should be interpreted by a qualified medical professional.
2. 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. 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 package label.

**IHC Protocol**
1. 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).
2. 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.
3. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
4. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
5. Wash slides with ImmunoDNA washer or DI water.
6. Continue IHC staining 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 |

**Abbreviated IF Protocol**

| Step | Incubation Time |
|---|---|
| Rinse slides in IF wash buffer | 5 minutes |
| Drain and wipe excess IF wash buffer off slide | |
| Conduct remaining steps in the dark | |
| Apply Antibody | 30-60 minutes |
| Rinse with 3 changes of IF wash buffer | 3x15 minutes each |
| Coverslip with IF mounting medium | |

**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 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 REP<br>QAdvis EAR AB<br>Ideon Science Park<br>Scheelevägen 17<br>SE-223 70 Lund, Sweden | Storage Temperature<br>Limites de température<br>Zulässiger Temperaturbereich | Manufacturer<br>Fabricant<br>Hersteller | REF<br>Catalog Number<br>Référence du catalogue<br>Bestellnummer |
| IVD<br>In Vitro Diagnostic Medical Device<br>Dispositif médical de diagnostic in vitro<br>In-Vitro-Diagnostikum | Read Instructions for Use<br>Consulter les instructions d'utilisation<br>Gebrauchsanweisung beachten | Expiration Date<br>Utiliser jusque<br>Verwendbar bis | LOT<br>Lot Number<br>Code du lot<br>Chargenbezeichnung |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