

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects mouse TIM-3 in direct ELISAs and Western blots. In direct ELISAs, approximately 10% cross-reactivity with recombinant human TIM-3 is observed and less than 5% cross-reactivity with recombinant mouse (rm) TIM-1, rmTIM-2, rmTIM-4, rmTIM-5, rmTIM-6, and rmTIM-7 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant mouse TIM-3 Leu22-Arg191 Accession # AAL65156
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

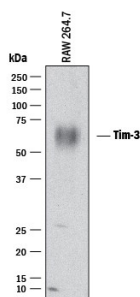
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. [General Protocols](#) are available in the Technical Information section on our website.

	Recommended Concentration	Sample
Western Blot	2 µg/mL	See Below
Flow Cytometry	2.5 µg/10 ⁶ cells	HT-2 mouse T cell line
Immunohistochemistry	3-15 µg/mL	Immersion fixed paraffin-embedded sections of mouse spleen
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

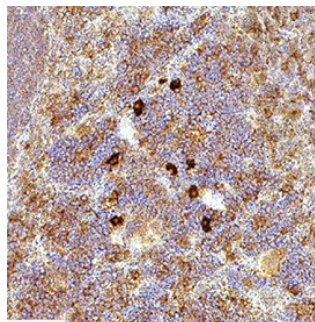
DATA

Western Blot



Detection of Mouse TIM-3 by Western Blot. Western blot shows lysates of RAW 264.7 mouse monocyte/macrophage cell line. PVDF membrane was probed with 2 µg/mL of Goat Anti-Mouse TIM-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1529) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for TIM-3 at approximately 45-70 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



TIM-3 in Mouse Spleen. TIM-3 was detected in immersion fixed paraffin-embedded sections of mouse spleen using Goat Anti-Mouse TIM-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1529) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes. Staining was performed using our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> 12 months from date of receipt, -20 to -70 °C as supplied. 1 month, 2 to 8 °C under sterile conditions after reconstitution. 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

TIM-3 (T cell immunoglobulin and mucin domain-3) is a 60 kDa member of the TIM family of immune regulating molecules. TIMs are type I transmembrane glycoproteins with one Ig-like V-type domain and a Ser/Thr-rich mucin stalk (1-3). There are three TIM genes in human and eight in mouse. Mature mouse TIM-3 consists of a 174 amino acid (aa) extracellular domain (ECD), a 21 aa transmembrane segment (TM), and a 67 aa cytoplasmic tail (4). Two alternately spliced isoforms have been reported in mouse which lack either the TM or both the TM and mucin regions (5, 6). Within the ECD, mouse TIM-3 shares 58% and 74% aa sequence identity with human and rat TIM-3, respectively. TIM-3 is specifically expressed on Th1 cells whereas TIM-1 and TIM-2 are expressed on Th2 cells. In chronic inflammation, autoimmune disorders, and some cancers, TIM-3 is upregulated on several other hematopoietic cell types and on hippocampal neurons (9-12). The glycosylated Ig domain of TIM-3 binds cell-associated galectin-9 which induces TIM-3 Tyr phosphorylation and proapoptotic signaling (10, 13). TIM-3 functions as a negative regulator of Th1 cell activity. Its blockade results in increased IFN- γ production, Th1 cell proliferation, and cytotoxicity (5, 7, 12, 14). TIM-3 may play a role in regulatory T cell development (7), inflammation (15), and immune tolerance (5, 13, 14). Soluble mouse TIM-3 has been shown to inhibit anti-tumor effector T cell responses and to enhance autoimmune reactions (6, 7).

References:

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