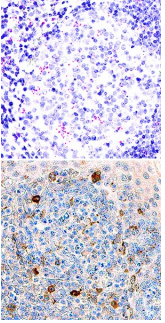
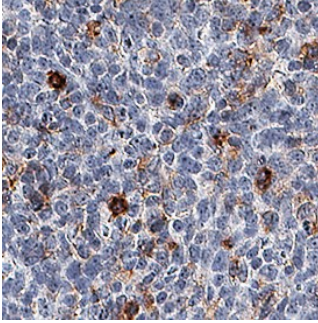
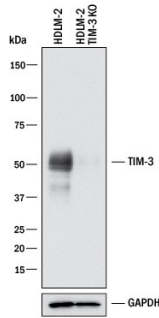


DESCRIPTION	
Species Reactivity	Human
Specificity	Detects human TIM-3 in direct ELISAs and Western blots. In direct ELISAs, approximately 5% cross-reactivity with recombinant mouse (rm) TIM-3 is observed and less than 1% cross-reactivity with recombinant human TIM-1, rmTIM-1, and rmTIM-2 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human TIM-3 Ser22-Arg200 Accession # Q8TDQ0
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		
<i>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.</i>		
	Recommended Concentration	Sample
Dual RNAscope ISH-IHC Compatible	3-25 µg/mL	See Below
Western Blot	0.1 µg/mL	Recombinant Human TIM-3 Fc Chimera (Catalog # 2365-TM)
Flow Cytometry	0.25 µg/10 ⁶ cells	Human peripheral blood monocytes
Immunohistochemistry	3-15 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Knockout Validated	TIM-3 is specifically detected in HDLM-2 human Hodgkin's lymphoma parental cell line but is not detectable in TIM-3 knockout HDLM-2 human Hodgkin's lymphoma cell line.	

DATA	
<p>Dual RNAscope ISH-IHC Compatible</p>  <p>TIM-3 in Human Tonsil Using Dual RNAscope[®]ISH and IHC. TIM-3 mRNA was detected in formalin-fixed paraffin-embedded tissue sections of human tonsil probed with ACD RNAscope[®]Probe (Catalog # 560681) and stained using ACD RNAscope[®]2.5 HD Detection Reagents-Red (top image, Catalog # 32260). Adjacent tissue section was processed for immunohistochemistry using R&D Systems Goat Anti-Human TIM-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2365) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte HRP Polymer Antibody (R&D Systems, Catalog # VC004) and DAB chromogen (lower image, yellow-brown). Tissues were counterstained with hematoxylin (blue).</p>	<p>Immunohistochemistry</p>  <p>TIM-3 in Human Tonsil. TIM-3 was detected in immersion fixed paraffin-embedded sections of human tonsil using Goat Anti-Human TIM-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2365) 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 cell membranes and extracellular space. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.</p>

Knockout Validated



Western Blot Shows Human TIM-3 Specificity by Using Knockout Cell Line.

Western blot shows lysates of HDLM-2 human Hodgkin's lymphoma cell line and human TIM-3 knockout HDLM-2 human Hodgkin's lymphoma cell line (KO). PVDF membrane was probed with 1 µg/mL of Goat Anti-Human TIM-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2365) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for TIM-3 at approximately 50 kDa (as indicated) in the parental HDLM-2 human Hodgkin's lymphoma cell line, but is not detectable in knockout HDLM-2 human Hodgkin's lymphoma cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

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 human TIM-3 consists of a 181 amino acid (aa) extracellular domain (ECD), a 21 aa transmembrane segment, and a 78 aa cytoplasmic tail (4). An alternately spliced isoform is truncated following a short substitution after the Ig-like domain. Within the ECD, human TIM-3 shares 58% aa sequence identity with mouse and rat TIM-3. TIM-3 is expressed on the surface of effector T cells (CD4⁺ Th1 and CD8⁺ Tc1) but not on helper T cells (CD4⁺ Th2 and CD8⁺ Tc2) (4, 5). In chronic inflammation, autoimmune disorders, and some cancers, TIM-3 is upregulated on several other hematopoietic cell types. It also occurs on hippocampal neurons (7-10). The Ig domain of TIM-3 interacts with a ligand on resting but not activated Th1 and Th2 cells (5, 11). The glycosylated Ig domain of TIM-3 binds cell-associated galectin-9. This induces TIM-3 Tyr phosphorylation and pro-apoptotic signaling (8, 12). 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, 10, 11, 13), regulatory T cell development (5), and increases in macrophage and neutrophil infiltration into sites of inflammation (14). Soluble mouse TIM-3 constructs which lack the cytoplasmic domain have been shown to inhibit anti-tumor effector T cell responses and to enhance autoimmune reactions (5, 15).

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