abcam

Product datasheet

Anti-TIM 3 antibody [EPR22241] ab241332

Recombinant RabMAb

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Overview

Product name	Anti-TIM 3 antibody [EPR22241]
Description	Rabbit monoclonal [EPR22241] to TIM 3
Host species	Rabbit
Tested applications	**Suitable for:** mIHC, WB, IP, IHC-P **Unsuitable for:** Flow Cyt or ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Daudi and RAW 264.7 whole cell lysates (+/- treatment with PNGase F). IP: Daudi whole cell lysate. IHC-P: Human liver and lung cancer tissue; mouse spleen tissue; rat spleen tissue. mIHC: Human tonsil
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR22241

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab241332 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
mIHC		Use at an assay dependent concentration.
WB	★★★★☆ (1)	1/1000. Detects a band of approximately 50-60 kDa (predicted molecular weight: 33 kDa).
IP		1/30.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

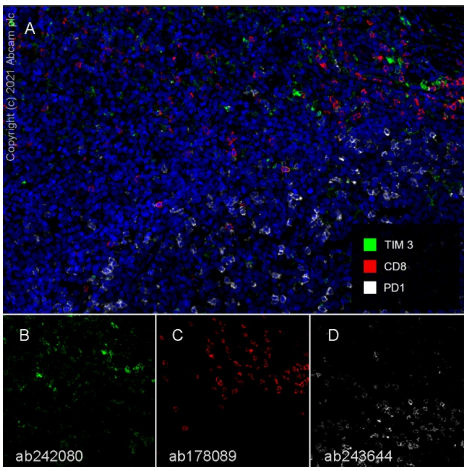
Application notes Is unsuitable for Flow Cyt or ICC/IF.

Target

Function Cell surface receptor implicated in modulating innate and adaptive immune responses. Generally accepted to have an inhibiting function. Reports on stimulating functions suggest that the activity may be influenced by the cellular context and/or the respective ligand (PubMed:24825777). Regulates macrophage activation (PubMed:11823861). Inhibits T-helper type 1 lymphocyte (Th1)-mediated auto- and alloimmune responses and promotes immunological tolerance (PubMed:14556005). In CD8+ cells attenuates TCR-induced signaling, specifically by blocking NF-kappaB and NFAT promoter activities resulting in the loss of IL-2 secretion. The function may implicate its association with LCK proposed to impair phosphorylation of TCR subunits, and/or LGALS9-dependent recruitment of PTPRC to the immunological synapse (PubMed:24337741, PubMed:26492563). In contrast, shown to activate TCR-induced signaling in T-cells probably implicating ZAP70, LCP2, LCK and FYN (By similarity). Expressed on Treg cells can inhibit Th17 cell responses (PubMed:24838857). Receptor for LGALS9 (PubMed:16286920, PubMed:24337741). Binding to LGALS9 is believed to result in suppression of T-cell responses; the resulting apoptosis of antigen-specific cells may implicate HAVCR2 phosphorylation and disruption of its association with BAG6. Binding to LGALS9 is proposed to be involved in innate immune response to intracellular pathogens. Expressed on Th1 cells interacts with LGALS9 expressed on Mycobacterium tuberculosis-infected macrophages to stimulate antibactericidal activity including IL-1 beta secretion and to restrict intracellular bacterial growth (By similarity). However, the function as receptor for LGALS9 has been challenged (PubMed:23555261). Also reported to enhance CD8+ T-cell responses to an acute infection such as by Listeria monocytogenes (By similarity). Receptor for phosphatidylserine (PtSer); PtSer-binding is calcium-dependent. May recognize PtSer on apoptotic cells leading to their phagocytosis. Mediates the engulfment of apoptotic cells by dendritic cells. Expressed on T-cells, promotes conjugation but not engulfment of apoptotic cells. Expressed on dendritic cells (DCs) positively regulates innate immune response and in synergy with Toll-like receptors promotes secretion of TNF-alpha. In tumor-imfiltrating DCs suppresses nucleic acid-mediated innate immune repsonse by interaction with HMGB1 and interfering with nucleic acid-sensing and trafficking of nucleid acids to

	endosomes (By similarity). Expressed on natural killer (NK) cells acts as a coreceptor to enhance IFN-gamma production in response to LGALS9 (PubMed:22323453). In contrast, shown to suppress NK cell-mediated cytotoxicity (PubMed:22383801). Negatively regulates NK cell function in LPS-induced endotoxic shock.
Tissue specificity	Expressed in T-helper type 1 (Th1) lymphocytes. Expressed on regulatory T (Treg) cells after TCR stimulation. Expressed in dendritic cells and natural killer (NK) cells. Expressed in epithelial tissues. Expression is increased on CD4+ and CD8+ T-cells in chronic hepatitis C virus (HCV) infection. In progressive HIV-1 infection, expression is up-regulated on HIV-1-specific CD8 T-cells.
Involvement in disease	May be involved in T-cell exhaustion associated with chronic viral infections such as with human immunodeficiency virus (HIV) and hepatitic C virus (HCV).
Sequence similarities	Belongs to the immunoglobulin superfamily. TIM family. Contains 1 Ig-like V-type (immunoglobulin-like) domain.
Post-translational modifications	O-glycosylated with core 1 or possibly core 8 glycans. Phosphorylated on tyrosine residues; modestly increased after TCR/CD28 stimulation. Can be phosphorylated in the cytoplasmatic domain by FYN (By similarity). Phosphorylation at Tyr-265 is increased by stimulation with ligand LGALS9.
Cellular localization	Membrane. Cell junction. Localizes to the immunological synapse between CD8+ T-cells and target cells.

Images



Multiplex immunohistochemistry - Anti-TIM 3 antibody [EPR22241] (ab241332)

This data was developed using **ab242080**, the same antibody clone in a different buffer formulation.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human tonsil labelling PD1 with **ab243644** at 1/500 dilution (1.02 µg/mL) (D), CD8 with **ab178089** at 1/100 dilution (0.83 µg/ml) (C) and TIM 3 with **ab242080** at 1/500 dilution (2.09 µg/ml) (B). Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

Panel A: merged staining of anti-TIM 3 (green; Opal™690), anti-CD8 (red; Opal™520) and anti-PD1 (gray; Opal™570) on human tonsil.

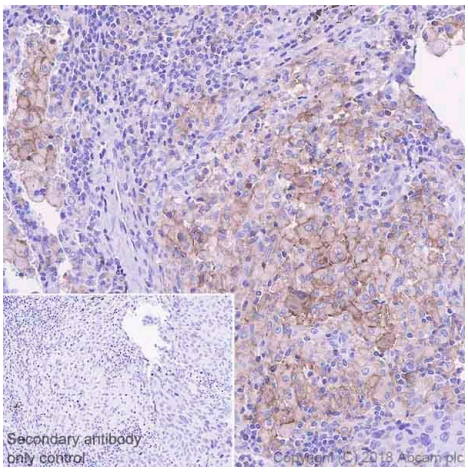
Panel B: anti-TIM 3 stained on membrane of a subset of immune cells.

Panel C: anti-CD8 stained on membrane of a subset of T cells.

Panel D: anti-PD1 stained on membrane of a subset of lymphocytes.

The section was incubated in three rounds of staining: in the order of **ab242080**, **ab243644** and **ab178089** for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

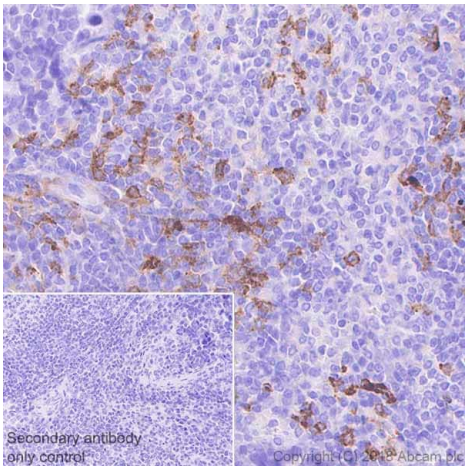


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TIM 3 antibody [EPR22241] (ab241332)

Immunohistochemical analysis of paraffin-embedded human lung cancer tissue labeling TIM 3 with ab241332 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Cytoplasmic and membranous staining on both infiltrated immunocytes and tumor cells of human lung cancer (PMID: 22586058) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

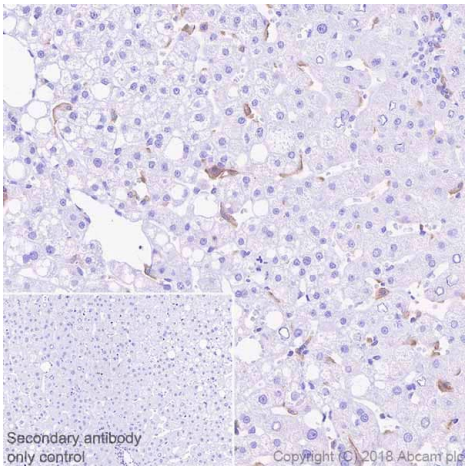


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TIM 3 antibody [EPR22241] (ab241332)

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling TIM 3 with ab241332 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Cytoplasmic and membranous staining on subsets of immune cells of mouse spleen is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

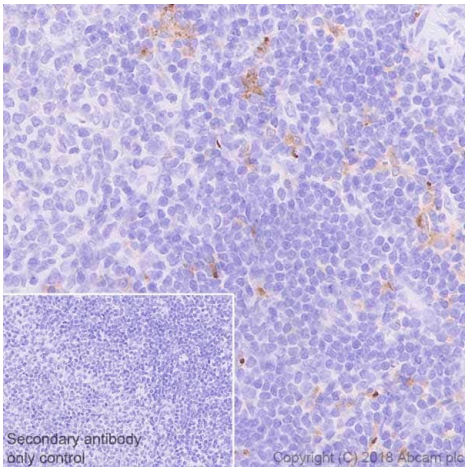


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TIM 3 antibody [EPR22241] (ab241332)

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling TIM 3 with ab241332 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Cytoplasmic and membranous staining on Kupffer cells of human liver (PMID: 27192565) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

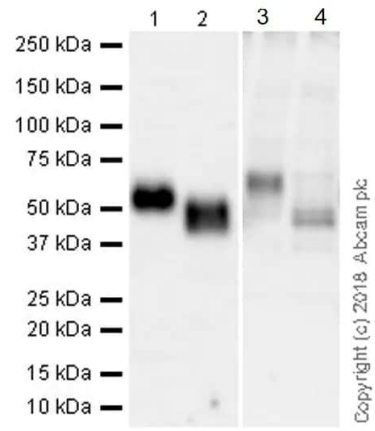


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TIM 3 antibody [EPR22241] (ab241332)

Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling TIM 3 with ab241332 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Cytoplasmic and membranous staining on subsets of immune cells of rat spleen is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Western blot - Anti-TIM 3 antibody [EPR22241] (ab241332)

All lanes : Anti-TIM 3 antibody [EPR22241] (ab241332) at 1/1000 dilution

Lane 1 : Daudi (human Burkitt's lymphoma cell line) whole cell lysate at 20 µg
Lane 2 : Daudi whole cell lysate (treated with PNGase F) at 20 mg/ml
Lane 3 : RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate at 20 µg
Lane 4 : RAW 264.7 whole cell lysate (treated with PNGase F) at 20 µg

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

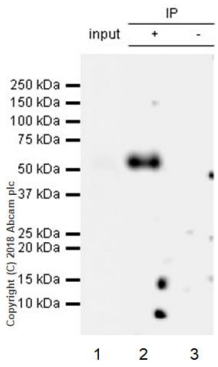
Developed using the ECL technique.

Predicted band size: 33 kDa
Observed band size: 40 kDa
Additional bands at: 50-60 kDa (possible glycosylated form)

Exposure time: 70 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

Lanes 3 & 4 were developed using a higher sensitivity ECL substrate.

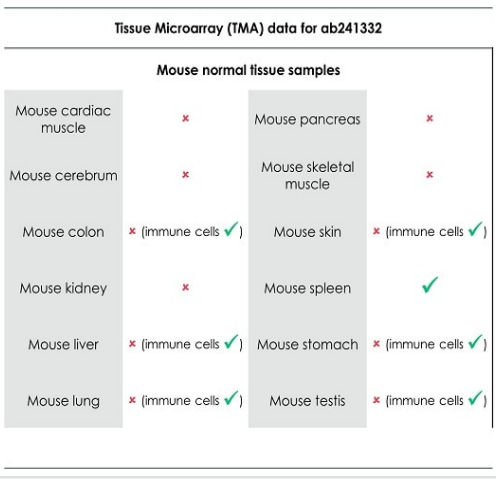


Immunoprecipitation - Anti-TIM 3 antibody [EPR22241] (ab241332)

TIM 3 was immunoprecipitated from 0.35 mg of Daudi (human Burkitt's lymphoma cell line) whole cell lysate with ab241332 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab241332 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

Lane 1: Daudi lysate 10 µg (Input).
Lane 2: ab241332 IP in Daudi whole cell lysate (+).
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab241332 in Daudi whole cell lysate (-).

Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 3 minutes.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TIM 3 antibody [EPR22241] (ab241332)

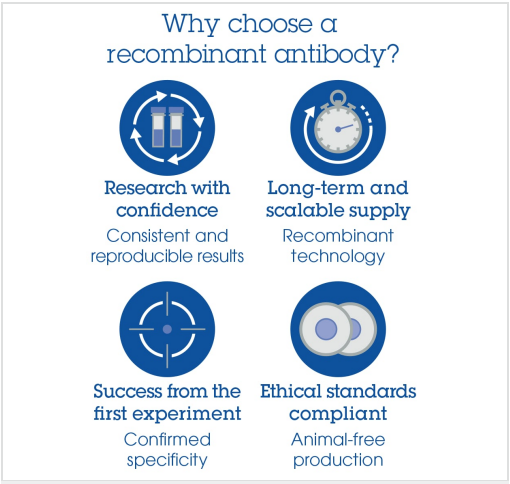
Tissue Microarrays stained for "Anti-TIM 3 antibody [EPR22241]" using "ab241332" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). The sections were incubated with ab241332 at +4°C overnight followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).

Tissue Microarray (TMA) data for ab241332

Human normal tissue samples				Human malignant tissue samples			
Human cardiac muscle	x	Human placenta	x (immune cells ✓)	Clear cell carcinoma of human kidney	x (immune cells ✓)	Human glioma	✓
Human cerebrum	x	Human skeletal muscle	x (immune cells ✓)	Human bladder cancer	x (immune cells ✓)	Human hepatocellular carcinoma	x (immune cells ✓)
Human colon	x (immune cells ✓)	Human skin	x (immune cells ✓)	Human breast carcinoma	x (immune cells ✓)	Human lung carcinoma	x (immune cells ✓)
Human endometrium	x (immune cells ✓)	Human spleen	✓	Human cervical carcinoma	x (immune cells ✓)	Human ovarian carcinoma	x (immune cells ✓)
Human kidney	x	Human stomach	x (immune cells ✓)	Human colon carcinoma	x (immune cells ✓)	Human pancreatic carcinoma	x (immune cells ✓)
Human liver	x (Kupffer cells ✓)	Human testis	x (immune cells ✓)	Human endometrial carcinoma	x (immune cells ✓)	Human prostatic hyperplasia	x (immune cells ✓)
Human lung	x (immune cells ✓)	Human thyroid	x (immune cells ✓)	Human gastric adenocarcinoma	x (immune cells ✓)	Human thyroid carcinoma	x (immune cells ✓)
Human mammary gland	x (immune cells ✓)	Human tonsil	✓				
Human pancreas	x (immune cells ✓)						

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TIM 3 antibody [EPR22241] (ab241332)

Tissue Microarrays stained for "Anti-TIM 3 antibody [EPR22241]" using "ab241332" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). The sections were incubated with ab241332 at +4°C overnight followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).



Anti-TIM 3 antibody [EPR22241] (ab241332)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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