CD366 (TIM-3) antibodies, human

For research use only

One test corresponds to labeling of up to 10^7 cells in a total volume of 100 µL.

Product	Content	Order no.
CD366 (TIM-3)-VioBright FITC	for 30 tests	130-109-758
CD366 (TIM-3)-VioBright FITC	for 100 tests	130-109-711
CD366 (TIM-3)-PE	for 30 tests	130-109-761
CD366 (TIM-3)-PE	for 100 tests	130-109-714
CD366 (TIM-3)-APC	for 30 tests	130-109-760
CD366 (TIM-3)-APC	for 100 tests	130-109-713
CD366 (TIM-3)-PE-Vio770	for 30 tests	130-109-759
CD366 (TIM-3)-PE-Vio770	for 100 tests	130-109-712
CD366 (TIM-3)-Biotin	for 30 tests	130-109-762
CD366 (TIM-3)-Biotin	for 100 tests	130-109-715

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Technical data and background information

Antigen	CD366 (TIM-3)
Clone	REA635
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	HAVcr-2, TIMD-3
Molecular mass of antigen [kDa]	31
Cross-reactivity	rhesus monkey (*Macaca mulatta*)
Distribution of antigen	dendritic cells, macrophages, monocytes, T cells
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA635 recognizes the human CD366 antigen, a single-pass type I membrane protein, which is also known as T cell immunoglobulin and mucin domain containing protein-3 (TIM-3) or Hepatitis A virus cellular receptor 2 (HAVcr-2). CD366 is expressed on activated T cells, especially Tн1 cells,

monocytes, macrophages, and dendritic cells. It is an activation-induced inhibitory molecule involved in tolerance and shown to induce T cell exhaustion in chronic viral infection and cancers. CD366 has been proposed to inhibit TH1-mediated immune responses and regulates TH1 and TH17 cytokine secretion. The interaction of CD366 and its ligand galectin-9 induces apoptosis of TH1 cells.
Additional information: Clone REA635 displays negligible binding to Fc receptors.

Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2−8 °C).
  Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

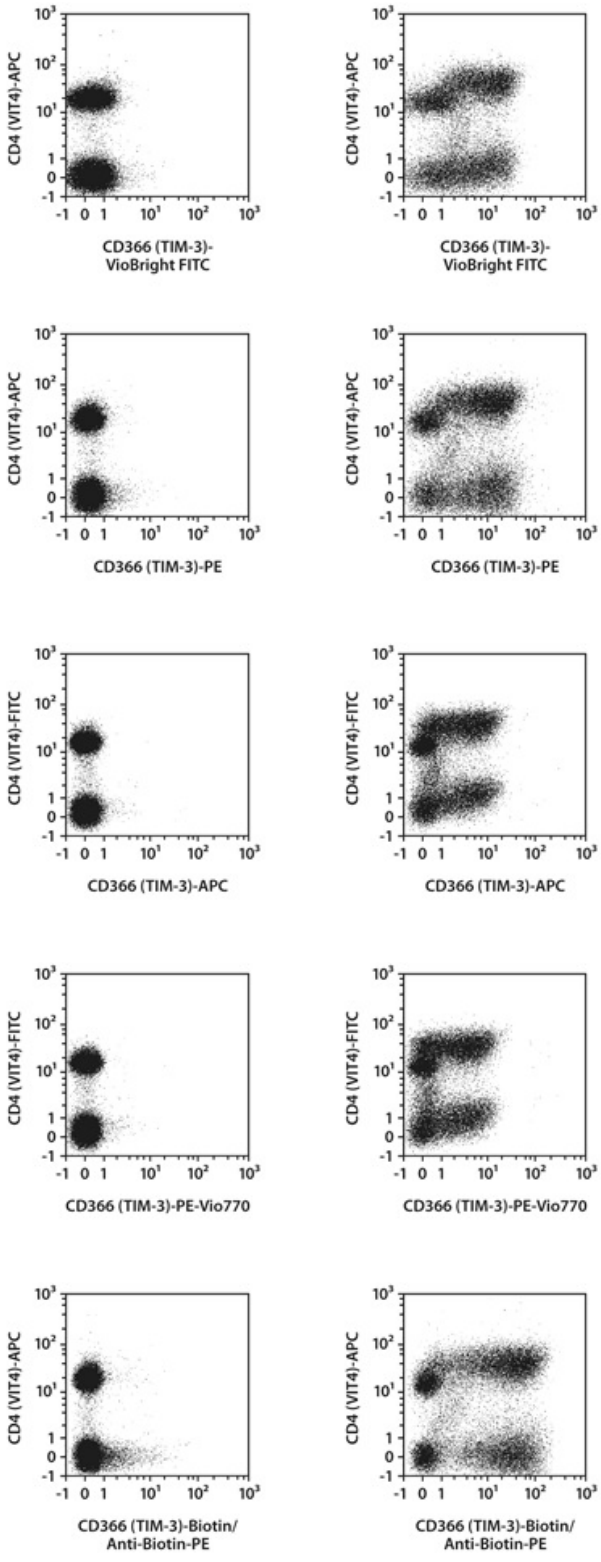
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1−2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of fluorochrome-conjugated anti-biotin antibody, and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

Examples of immunofluorescent staining

Human peripheral blood mononuclear cells (PBMCs), either left unstimulated (left image) or stimulated with CD3/CD28 antibodies for 3 days, were stained with CD366 (TIM-3) antibodies as well as with CD4 (VIT-4) antibodies. Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye–conjugated antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.



References

1. **Monney, L. *et al.*** (2002) Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. Nature 415: 536–541.
2. **Zhu, C. *et al.*** (2005) The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat. Immunol. 6(12): 1245–1252.
3. **Hastings, W. D. *et al.*** (2009) TIM-3 is expressed on activated human $CD4^+$ T cells and regulates Th1 and Th17 cytokines. Eur. J. Immunol. 39(9): 2492–2501.

Warranty

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