Anti-TIM-1 antibodies, mouse

For research use only

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10^6 cells.

Product	Content	Order no.
Anti-TIM-1-VioBright FITC	9 µg in 300 µL	130-110-429
Anti-TIM-1-VioBright FITC	30 µg in 1 mL	130-110-328
Anti-TIM-1-PE	9 µg in 300 µL	130-110-426
Anti-TIM-1-PE	30 µg in 1 mL	130-110-325
Anti-TIM-1-APC	9 µg in 300 µL	130-110-427
Anti-TIM-1-APC	30 µg in 1 mL	130-110-326
Anti-TIM-1-PE-Vio770	9 µg in 300 µL	130-110-428
Anti-TIM-1-PE-Vio770	30 µg in 1 mL	130-110-327
Anti-TIM-1-Biotin	9 µg in 300 µL	130-110-425
Anti-TIM-1-Biotin	30 µg in 1 mL	130-110-324

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	TIM-1
Clone	REA692
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	HAVcr-1, TIMD-1, KIM-1, TIM, TIM1
Molecular mass of antigen [kDa]	31
Distribution of antigen	T cells, kidney
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 REA692 recognizes the mouse T cell immunoglobulin mucin receptor 1 (TIM-1) antigen, a single-pass type I membrane protein, also known as hepatitis A virus cellular receptor 1 (HAVcr-1) or kidney injury molecule 1 (KIM-1). The TIM gene family is involved in a variety of immunity related processes including T cell proliferation and survival, tissue inflammation, and atopy. In mice, eight TIM genes encode the proteins TIM-1 to TIM-8, whereas only three TIM genes are found in humans

encoding TIM-1, TIM-3, and TIM-4. Several lines of evidence suggest that TIM-1 regulates T cell activity *in vivo* through responses mediated by TH1, TH2, TH17, and regulatory T cells. TIM-1 is recruited to the T cell receptor signaling complex and has a costimulatory role. TIM-1 expression has been demonstrated in epithelial cells, especially those of kidney origin, and is greatly increased in both mouse and human kidneys after injury. While absent on naive CD4 T cells, TIM-1 expression increases following TCR stimulation to provide positive co-stimulatory signal in T cell proliferation and TH1/TH17 cytokine production. It is reported, that the interaction of TIM-1 with TIM-4 is involved in the regulation of T cell proliferation.
Additional information: Clone REA692 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.

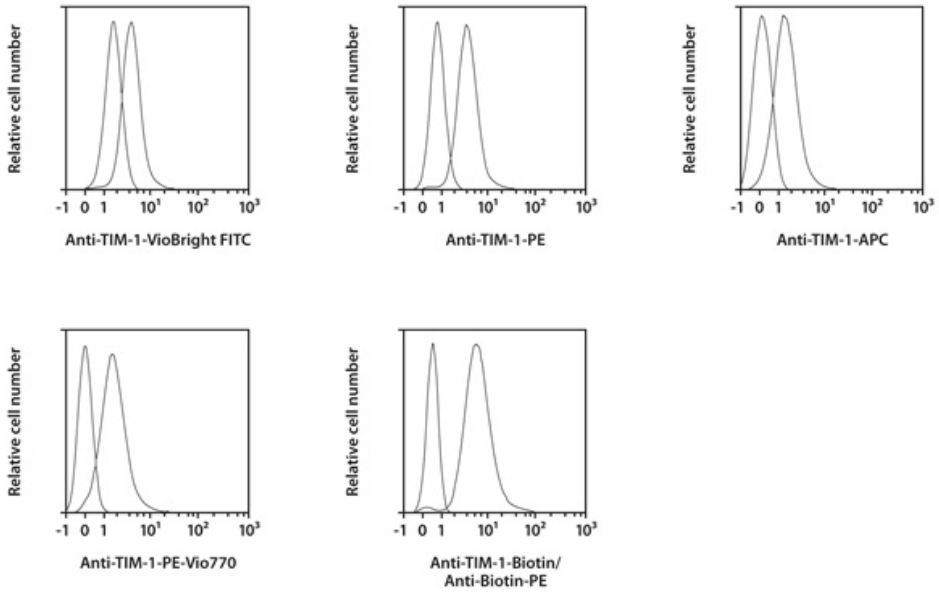
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10^6 cells/50 µL of buffer.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 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 2×10^6 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 10^6 nucleated cells per 45 µL of buffer.
4. Add 5 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

Mouse TIM-1 transfected mouse lymphoma L5178Y cells were stained with Anti-TIM-1 antibodies or with the corresponding REA Control antibodies (left peak) and analyzed by flow cytometry using the MACSQuant® Analyzer. 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. **Feigelstock, D. *et al.*** (1998) The human homolog of HAVcr-1 codes for a hepatitis A virus cellular receptor. J. Virol. 72(8): 6621–6628.
2. **Meyers, J. H. *et al.*** (2005) TIM-4 is the ligand for TIM-1, and the TIM-1-TIM-4 interaction regulates T cell proliferation. Nat. Immunol. 6(5): 455–464.
3. **Moller-Tank, S. *et al.*** (2013) Role of the phosphatidylserine receptor TIM-1 in enveloped-virus entry. J. Virol. 87(15): 8327–8341.
4. **Angiar, I. S. *et al.*** (2014) TIM-1 glycoprotein binds the adhesion receptor P-selectin and mediates T cell trafficking during inflammation and autoimmunity. Immunity 40(4): 542–553.

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