

Contents

1. Description
 - 1.1 Background information
 - 1.2 Applications
 - 1.3 Recommended antibody dilution
 - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
3. Example of immunofluorescent staining with Anti-TIGIT antibodies
4. Reference

1. Description

Components	1 mL monoclonal Anti-TIGIT antibodies, human conjugated to: Biotin 130-096-382
Clone	4E1.2 (isotype: mouse IgG3).
Capacity	100 tests or up to 10^9 total cells.
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

The clone 4E1.2 reacts with a protein containing an immunoglobulin variable (IgV) domain, a transmembrane domain, and an immunoreceptor tyrosine-based inhibitory motif (ITIM), which is called TIGIT (T cell immunoglobulin and ITIM domain). It is expressed on T cells, including Treg and memory subsets, as well as on NK cells. The poliovirus receptor (PVR; also called NECL5 or CD155) is a high-affinity coreceptor for TIGIT that is highly expressed on dendritic cells (DCs), fibroblasts, endothelial cells, and some tumor cells. The interaction of TIGIT with PVR on DCs leads to the downregulation of pro-inflammatory cytokine secretion and the induction of IL-10 production in DCs, executing an inhibitory effect on T cell activation.

1.2 Applications

- Identification and enumeration of TIGIT⁺ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for Anti-TIGIT-Biotin is **1:11 for up to 10^7 cells/100 μ L** of buffer for labeling of cells and subsequent analysis by flow cytometry.

Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

1.4 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). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with Anti-TIGIT-Biotin.
- (Optional) CD3-FITC (# 130-080-401). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.

2. General protocol for immunofluorescent staining

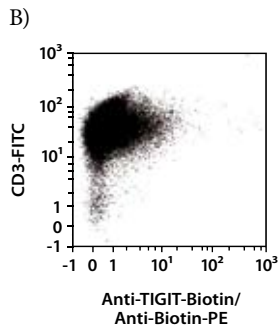
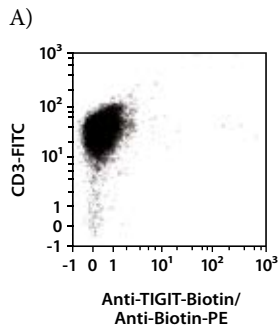
▲ Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated 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 μ L of buffer.
4. Add 10 μ L of the Anti-TIGIT 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 Anti-TIGIT-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of 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.

3. Example of immunofluorescent staining with Anti-TIGIT antibodies

Isolated CD4⁺ cells were activated by CD3 pure – functional grade (# 130-093-387) plate-bound and CD28 pure – functional grade (# 130-093-375) soluble antibodies for 72 hours. TIGIT expression was detected by Anti-TIGIT-Biotin and Anti-Biotin-PE as well as with CD3-FITC (# 130-080-401) 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.

Dot plot A shows TIGIT expression of unstimulated control and Dot plot B the expression of stimulated cells.



4. Reference

1. Yu, X. *et al.* (2008) The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. *Nature Immunology* 10: 48–57.

All protocols and data sheets are available at www.miltenyibiotec.com.

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.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

autoMACS, MACS, and MACSQuant are registered trademarks of Miltenyi Biotec GmbH.

Copyright © 2011 Miltenyi Biotec GmbH. All rights reserved.