

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 CD29 antibodies
4. References

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.

1. Description

This product is for research use only.

Components Monoclonal CD29 antibodies, human conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
FITC	130-101-256	130-101-255
PE	130-101-273	130-101-275
APC	130-101-280	130-101-271
PE-Vio770™	130-101-276	130-101-281
APC-Vio770™	130-101-259	130-101-261
Biotin	130-101-262	130-101-265

Clone TS2/16 (isotype: mouse IgG1κ).

Capacity 1 mL: 100 tests or up to 10⁹ total cells
300 µL: 30 tests or up to 3×10⁸ 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

- Antigen: CD29
- Synonym: integrin β1; GPIIa

- Expression patterns: Clone TS2/16 reacts with CD29, a 130 kDa single pass type I membrane protein. CD29 forms heterodimers with integrins α1-11 and αV to form functional integrin receptors which bind several cell surface and extracellular molecules such as collagen, fibronectin, laminin, VCAM1, cytotactin, osteopontin, epiligrin, thrombospondin, etc. The cytoplasmic domain of CD29 can interact with cytoskeletal proteins and signaling molecules. In humans, CD29 can be found in 4 different isoforms (β1A, β1B, β1C, β1D).

1.2 Applications

- Identification and enumeration of CD29⁺ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD29 conjugates is **1:11 for up to 10⁷ cells/100 µL** 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). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.

- (Optional) FcR Blocking Reagent, human (#130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (#130-090-756) as secondary antibody reagent in combination with CD29-Biotin.
- (Optional) For antibodies for additional staining or for isotype control, refer to www.miltenyibiotec.com/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.

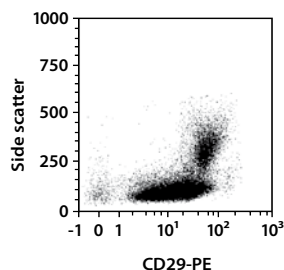
2. General protocol for immunofluorescent staining

Volumes given below are for **up to 10⁷** nucleated cells. When working with fewer than 10⁷ 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 2×10⁷ 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 10⁷ nucleated cells per 100 µL of buffer.
4. Add 10 µL of the CD29 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 CD29-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL 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 CD29 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD29 antibodies conjugated to PE 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.



For more examples please refer to the respective product page at www.miltenyibiotec.com/antibodies.

4. References

1. Brakebusch, C. *et al.* (1997) Genetic analysis of beta1 integrin function: confirmed, new and revised roles for a crucial family of cell adhesion molecules. *J. Cell. Sci.* 110: 2895–2904.
2. Altruda, F. *et al.* (1990) A human integrin beta 1 subunit with a unique cytoplasmic domain generated by alternative mRNA processing. *Gene* 95: 261–266.
3. Shih, D. T. *et al.* (1993) Structure/function analysis of the integrin beta 1 subunit by epitope mapping. *J. Cell Biol.* 122: 1361.

Refer to www.miltenyibiotec.com for all data sheets and protocols.

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, MACSQuant, and VioBlue are registered trademarks and VioGreen and Vio770 are trademarks of Miltenyi Biotec GmbH.

Copyright © 2013 Miltenyi Biotec GmbH. All rights reserved.