

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-Biotin antibodies

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 Anti-Biotin antibodies, conjugated to:

Conjugate	Order no. 200 µL (100 tests)	Order no. 60 µL (30 tests)
FITC	130-113-290	130-113-852
VioBright™ FITC	130-113-296	130-113-858
PE	130-113-291	130-113-853
APC	130-113-288	130-113-850
VioBlue®	130-113-295	130-113-857
VioGreen™	130-113-297	130-113-859
PerCP	130-113-293	130-113-855
PE-Vio® 615	130-114-543	130-114-734
PE-Vio® 770	130-113-292	130-113-854
APC-Vio® 770	130-113-289	130-113-851
PerCP-Vio® 700	130-113-294	130-113-856
Vio® 515	130-114-544	130-114-735
VioBright™ 515	130-113-298	130-113-860

Clone Bio3-18E7 (isotype: mouse IgG1).

Capacity 200 µL: 100 tests or up to 10⁸ total cells
60 µL: 30 tests or up to 3×10⁷ 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: Biotin
- Expression patterns: Anti-Biotin fluorochromes are ideal for fluorescent staining of cells separated with Anti-Biotin MicroBeads, Streptavidin MicroBeads, or with the Anti-Biotin MultiSort Kit. They can also be used to stain cells labeled with cocktails of biotinylated antibodies, for example for quality control when using MACS® Cell Isolation Kits for the isolation of untouched cells. The Anti-Biotin antibody does not bind to free biotin which is often present in cell culture media.

1.2 Applications

- Fluorescent staining of cells labeled with a biotinylated primary antibody.
- Fluorescent staining of cells separated with Anti-Biotin MicroBeads (# 130-090-485) or Streptavidin MicroBeads (# 130-048-101).
- Quality control of separations using MACS Technology.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-Biotin conjugates is **1:50 for up to 10⁶ cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry. For Anti-Biotin MicroBead-labeled cells use the same dilution. Staining intensity depends on biotinylation grade of the primary antibody.

The antibody is suited for staining of formaldehyde-fixed cells.

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) Tandem Signal Enhancer, human (# 130-099-888) to reduce non-specific binding of tandem dye-conjugated antibodies to human cells, especially to monocytes.
- (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 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).

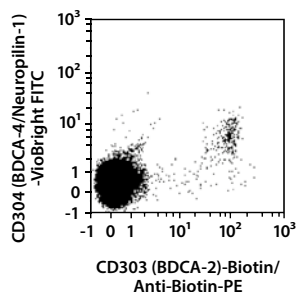
1. Determine cell number.
2. Centrifuge cell suspension at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 μL of buffer.
4. Add 2 μL of the Anti-Biotin antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ($2-8^\circ\text{C}$).

▲ **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 $300 \times g$ for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Example of immunofluorescent staining with Anti-Biotin antibodies

Human peripheral blood mononuclear cells (PBMCs) were labeled with CD303 (BDCA-2)-Biotin and fluorescently stained with Anti-Biotin-PE antibodies. Cells were counterstained with CD304 (BDCA-4/Neuropilin-1)-VioBright™ FITC and analyzed by flow cytometry using the MACSQuant® Analyzer.



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

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, the MACS logo, MACSQuant, Vio, VioBlue, VioBright, and VioGreen are registered trademarks or trademarks of Miltenyi Biotec GmbH and/or its affiliates in various countries worldwide.

Copyright © 2018 Miltenyi Biotec GmbH and/or its affiliates. All rights reserved.