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## 1. Description

**Components** 1 mL monoclonal Anti-Biotin antibodies conjugated to various dyes.

|            |             |
|------------|-------------|
| FITC       | 130-090-857 |
| PE         | 130-090-756 |
| APC        | 130-090-856 |
| VioBlue®   | 130-094-669 |
| VioGreen™  | 130-097-022 |
| PerCP      | 130-094-974 |
| PE-Vio770™ | 130-096-632 |
| APC-Vio770 | 130-096-630 |

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

**Capacity** 100 tests or up to 10<sup>9</sup> 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

Anti-Biotin fluorochromes are perfectly suitable for indirect immunofluorescent staining when working with a biotinylated primary antibody. They can also be used for simultaneous staining of several cell surface markers labeled with a cocktail of biotinylated antibodies. 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 **1:11 for up to 10<sup>7</sup> cells/100 µL** of buffer for labeling of cells and 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 formaldehyd-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). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.

- (Optional) Anti-Biotin MicroBeads (# 130-090-485) for magnetic separation of cells labeled with biotinylated primary antibodies.
- (Optional) CD304 (BDCA-4/Neuropilin-1)-PE (# 130-090-533), CD304 (BDCA-4/Neuropilin-1)-APC (# 130-090-900), or CD303 (BDCA-2)-Biotin (# 130-090-691). For more information about antibodies refer to [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies).
- (Optional) Mouse IgG1 isotype control antibodies conjugated to, e.g., VioBlue (# 130-094-670). For more information about isotype control antibodies refer to [www.miltenyibiotec.com](http://www.miltenyibiotec.com).
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD 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. Protocol

### 2.1 Labeling of cells with primary biotinylated antibodies

1. Label cells with biotinylated antibody at time and titer recommended by the manufacturer. Typically, labeling for 10 minutes is sufficient.

▲ **Note:** The biotinylated antibody should be used at its optimal titer, i.e., with optimal labeling intensity and no background labeling.

2. Wash cells by adding 1–2 mL of buffer per 10<sup>7</sup> cells and centrifuge for 10 minutes at 300×g and 4 °C.

▲ **Note:** The optimal relative centrifugal force (RCF) and centrifugation time may be different depending on the cell sample.

- Carefully remove the supernatant completely.
- (Optional) Repeat steps 2 and 3.

▲ **Note:** Wash cells carefully after incubation with primary antibody to remove unbound primary antibody.

- (Optional) Perform magnetic labeling and magnetic separation with Anti-Biotin MicroBeads according to the Anti-Biotin MicroBead data sheet.

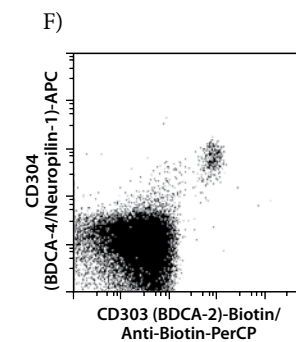
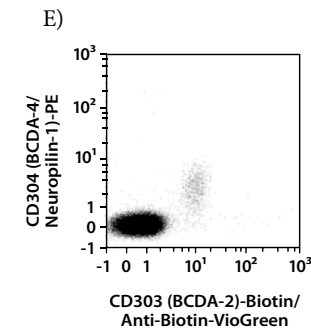
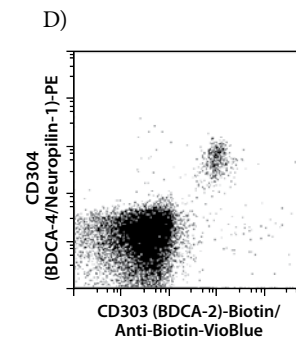
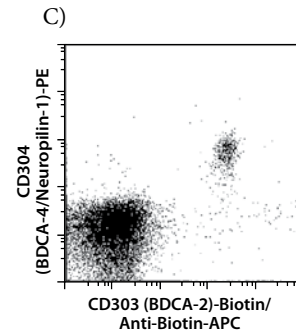
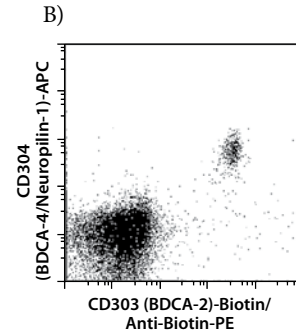
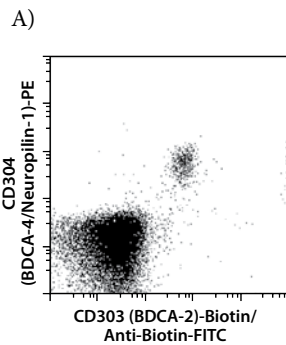
## 2.2 Fluorescent staining of cells labeled with primary biotinylated antibodies

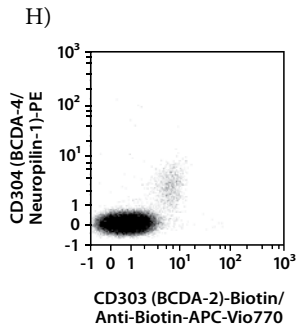
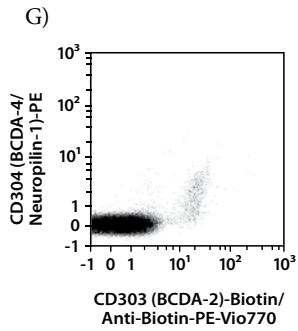
▲ Volumes given below are for up to  $10^7$  nucleated cells. When working with fewer than  $10^7$  cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for  $2 \times 10^7$  nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

- Determine cell number.
- Centrifuge cell suspension at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
- Resuspend up to  $10^7$  nucleated cells per 100  $\mu$ L of buffer.
- Add 10  $\mu$ L of the Anti-Biotin antibody.
- 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.
- Wash cells by adding 1–2 mL of buffer and centrifuge at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
- Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

## 3. Examples of immunofluorescent staining with Anti-Biotin antibodies

Human peripheral blood mononuclear cells (PBMCs) were labeled with biotinylated CD303 (BDCA-2) antibodies and fluorescently stained with CD304 (BDCA-4/Neuropilin-1) conjugated to PE or APC and Anti-Biotin antibodies conjugated to FITC (A), PE (B), APC (C), VioBlue (D), VioGreen (E), PerCP (F), PE-Vio770 (G), or APC-Vio770 (H). Cells were analyzed by flow cytometry using the MACSQuant<sup>®</sup> Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.





All protocols and data sheets are available at [www.miltenyibiotec.com](http://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

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