Labeling Check Reagent

Miltenyi Biotec

Contents

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 Labeling Check Reagent conjugated to:

Conjugate	Order no. 200 µL (100 tests)	Order no. 60 µL (30 tests)
PE	130-119-773	130-119-813
APC	130-122-219	130-122-228
VioBlue®	130-116-501	130-116-659
FITC	130-124-695	130-124-701

Clone AC146 (isotype: mouse IgG3, κ).

Capacity 200 µL: 100 tests or up to 10^8 total cells

60 µL: 30 tests or up to 3×10^7 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 Labeling Check Reagent has been developed for the flow cytometric detection of cells of all species labeled with MACS® MicroBeads or MACSiBeads™ Particles.

1.2 Applications

- Staining of cells separated by MACS Technology.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Labeling Check Reagent conjugates is **1:50 for up to 10^6 cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry.

The antibody is suited for staining of formaldehyde-fixed cells. 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 respective serum albumin, respective serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- FcR Blocking Reagent, mouse (# 130-092-575) or FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.
- (Optional) A list of antibodies for additional staining is available at www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells without fixation.

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×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.

   ▲ **Note:** If FcR Blocking Reagent, mouse is being used add 88 µL of buffer and 10 µL of the FcR Blocking Reagent, mouse directly before addition of the Labeling Check Reagent per 10^6 nucleated cells.

   ▲ **Note:** If FcR Blocking Reagent, human is being used add 78 µL of buffer and 20 µL of the FcR Blocking Reagent, human directly before addition of the Labeling Check Reagent per 10^6 nucleated cells.

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4. Add 2 µL of the Labeling Check Reagent.

5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

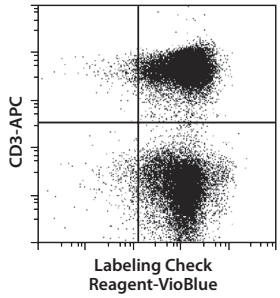
   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry.

3. Examples of immunofluorescent staining with Labeling Check Reagent

$CD56^+$ cells were isolated from human peripheral blood mononuclear cells (PBMCs) using CD56 MicroBeads (# 130-050-401). Cells were fluorescently stained with Labeling Check Reagent as well as with CD3 antibodies before and after separation. Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on the scatter signals and propidium iodide fluorescence.



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.