Miltenyi Biotec

Hematopoietic Lineage Labeling Cocktail, anti-mouse, Biotin

Order no. 130-092-613

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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

Components	**1 mL Hematopoietic Lineage Labeling Cocktail, anti-mouse, Biotin** containing:
	CD5 Antibody, anti-mouse, Biotin (clone: 53-7.3, isotype: rat IgG2aκ)
	CD11b Antibody, anti-human/mouse, Biotin (clone: M1/70.15.11.5, isotype: rat IgG2bκ)
	CD45R (B220) Antibody, anti-mouse, Biotin (clone: RA3-6B2, isotype: rat IgG2aκ)
	Ly-6B.2 Antibody, anti-mouse, Biotin, REAfinity™ (clone: REA115, isotype: recombinant human IgG1)
	Gr-1 Antibody, anti-mouse, Biotin (clone: RB6-8C5, isotype: rat IgG2bκ)
	Ter-119 Antibody, anti-mouse, Biotin (clone: Ter-119, isotype: rat IgG2bκ)
Capacity	100 tests or up to 10^8 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 Hematopoietic Lineage Labeling Cocktail, anti-mouse, Biotin is designed for labeling of mouse cells committed to hematopoietic lineage differentiation. It contains a panel of monoclonal antibodies that recognize antigens on T cells, B cells, monocytes/macrophages, granulocytes, and erythrocytes. The Hematopoietic Lineage Labeling Cocktail, anti-mouse, Biotin is especially useful for labeling of lineage-committed, late hematopoietic progenitor cells residing in the bone marrow. Biotinylation of primary antibodies provides flexibility for secondary labeling with fluorochromes in immunofluorescent applications.

1.2 Applications

- Identification and enumeration of lineage$^+$ and lineage$^-$ cells from mouse bone marrow by flow cytometry.
- Enumeration of lineage$^+$ and lineage$^-$ cells by flow cytometry after MACS Separation of mouse bone marrow cells.

1.3 Reagent and instrument 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^{2+} or Mg^{2+} are not recommended for use.

- Flow cytometer equipped with a red (640 nm), a blue (488 nm), or a violet (405 nm) laser, e.g., MACSQuant Analyzer 10 (# 130-096-343) or MACSQuant Analyzer 16 (# 130-109-803).
- Biotin Antibody conjugated to FITC, PE, or APC, e.g., Biotin Antibody (clone: Bio3-18E7).
- (Optional) CD117 Antibody, anti-mouse conjugated to PE or APC, e.g., CD117 Antibody, anti-mouse, REAfinity (clone: REA791).
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells without fixation.
- (Optional) MACS Comp Bead Kit, anti-REA (# 130-104-693), anti-mouse Igκ (# 130-097-900), or anti-rat Igκ (# 130-107-755) for optimal compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.

2. Protocol

▲ For more information refer to the application protocol "Immunophenotyping of hematopoietic stem cells (HSCs) from mouse bone marrow using flow cytometry" at www.miltenyibiotec.com/applications.

▲ 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).

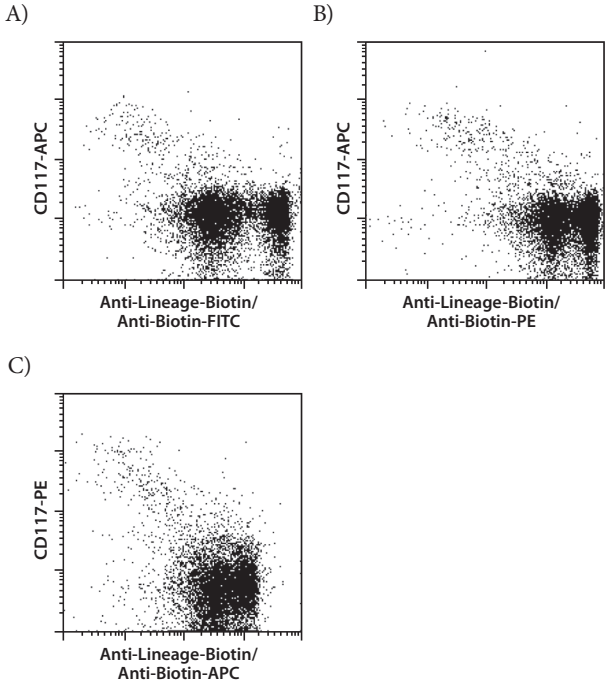
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1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 100 µL of buffer.
4. Add 10 µL of Hematopoietic Lineage Labeling Cocktail, anti-mouse, Biotin.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

   ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. Wash cells by adding 1–2 mL of buffer per 10^6 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Add Biotin Antibody in the dilution mentioned in the respective data sheet.
8. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
9. Wash cells by adding 1–2 mL of buffer per 10^6 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
10. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry.

3. Example of immunofluorescent staining with the Hematopoietic Lineage Labeling Cocktail, anti-mouse, Biotin

Mouse bone marrow cells were stained with Hematopoietic Lineage Labeling Cocktail, anti-mouse, Biotin as well as Biotin Antibody-FITC and CD117-APC (a), Biotin Antibody-PE and CD117-APC (b), or Biotin Antibody-APC and CD117-PE (c) and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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