Miltenyi Biotec

# MACS® Comp Mouse Igκ Reagent

Order no. 130-094-548

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## 1. Description

**Components** 1 mL MACS® Comp Mouse Igκ Reagent: monoclonal anti-human CD52 antibody (humanized IgG1, kappa) conjugated to anti-mouse κ monoclonal antibody (rat IgG1).

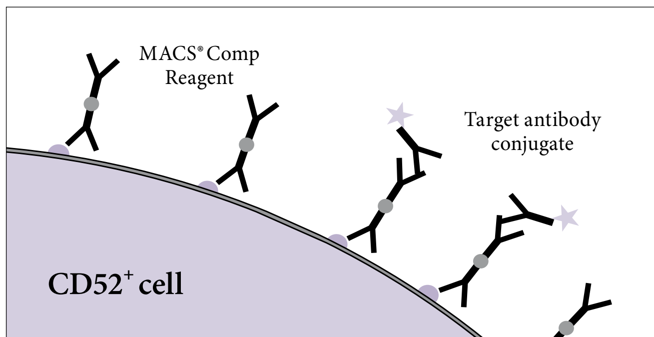
**Capacity** 100 tests or $10^8$ total cells.

**Product format** Antibody conjugates are supplied in a solution 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 Principle

The MACS® Comp Mouse Igκ Reagent is a dual antibody system that allows for the compensation of any given fluorochrome conjugated to mouse antibodies with κ light chains.
In contrast to bead-based compensation systems, the cell-based MACS Comp Igκ reagent system takes into account the autofluorescence of cells during compensation and avoids overcompensation. Equal quantities of stained and unstained cells are mixed and compensation is performed using a lymphocte gate.
The dual antibody system of the MACS Comp Mouse Igκ Reagent selectively targets CD52-expressing cells (all leukocytes). In order to perform compensation the second half of this dual antibody system also binds the κ light chain of the mouse antibody.




Human cells are incubated with the MACS® Comp Mouse Igκ Reagent for labeling via CD52 after which the fluorochrome-conjugated antibody in question is added. Thus, all CD52-positive cells within the sample are fluorescently labeled with the target antibody—irrespective of the antibody‘s specificity.
Unlabeled cells are spiked into the sample to serve as an internal negative control. Cells are analyzed by flow cytometry and compensation is performed using the fluorescence signal of the target antibody.

### 1.2 Application

- Efficient compensation for flow cytometric analysis.

### 1.3 Recommended reagent dilution

for labeling of human cells.

| | MACS® Comp Igκ[a] |
|---|---|
| Reagent dilution for flow cytometry | 1:11 |

a) Suitable for use with fresh or fixed cells, e.g. PBMCs, leukapheresis harvest, or lysed blood.

### 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 calf serum. Buffers or media containing $Ca^{2+}$ or $Mg^{2+}$ are not recommended for use.

- A minimum of $10^6$ freshly isolated human peripheral blood mononuclear cells (PBMCs). PBMCs should be isolated by density gradient centrifugation. For details see General Protocols in the User Manuals or visit www.miltenyibiotec.com.

  ▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

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## 2. General protocol for MACS® Comp Mouse Igκ Reagent use

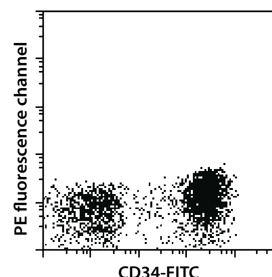
▲ Work fast, keep the cells cold, use pre-cooled solutions which will prevent capping of antibodies on the cell surface and a non-specific cell labeling.

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

1. Resuspend $5\times10^5$ PBMCs in 100 µL of buffer.
2. Add 10 µL of MACS® Comp Mouse Igκ Reagent.
3. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
4. Wash cells by adding 1−2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
5. Resuspend cells in 100 µL of buffer.
6. Add the antibody conjugate to be compensated in the recommended titer.
7. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
8. Wash cells by adding 1−2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. Resuspend cells in 500 µL of buffer.
10. **Immediately** before measurement, add a further $5\times10^5$ unstained PBMCs in 500 µL of buffer to serve as an internal negative control.
11. Proceed to compensation procedure. For automatic compensation, follow the instructions provided by the manufacturer of your flow cytometer. Manual compensation can be performed as outlined in the following steps.
12. Set a lymphocyte gate. In general, only cells with identical autofluorescent characteristics should be viewed.
13. Make sure that the voltage adjustment in each channel is optimized for unstained cells. Create a dot plot displaying the fluorescence channel of the used fluorochrome. Set the opposing axis to the appropriate fluorescence channel to eliminate fluorescence overlap (e.g. PE fluorescence channel for FITC-conjugated antibodies). Create separate regions for the analysis of positive and negative cells.
14. Open the statistics window to display the median fluorescence intensity of both populations. Adjust the compensation values in the channel to be corrected so that the median fluorescence intensities of the stained cell population equals that of the unstained cells.
15. Repeat steps 12−14 for compensation of additional antibody conjugates/fluorescence channels.

## 3. Example compensation with MACS® Comp Mouse Igκ Reagent

Human PBMCs were labeled with MACS® Comp Mouse Igκ Reagent followed by the addition of the fluorochrome-conjugated antibody to be compensated for. Unlabeled PBMCs were added directly before measurement. Data depicting the optimal compensation of CD34-FITC (# 130-081-001) are shown.




### 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.

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