MACS® Comp Reagent
mouse

MACS Comp Reagent-PE	130-092-670
MACS Comp Reagent-APC	130-092-668

Index

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.

Product format 1 mL MACS Comp Reagent, mouse: Anti-leukocyte CD45 monoclonal antibody (rat IgG2b) conjugated to anti-fluorochrome monoclonal antibody (mouse IgG1).

Antibody conjugates are supplied in a solution containing stabilizer and 0.05% sodium azide.

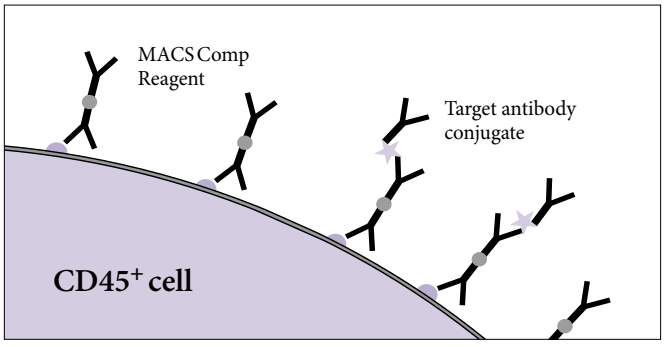
Product size 100 tests.

Storage Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle and product applications

The MACS Comp Reagent is a dual antibody system that allows for compensation of PE- or APC-conjugated antibodies and several tandem fluorochrome conjugates. For a list, please see the table in section 1.2.

In contrast to bead-based compensation systems, the cell-based MACS Comp 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 lymphocyte gate.



The MACS Comp Reagent employs a dual antibody system that selectively targets CD45-expressing cells (all leukocytes) as well as the fluorochrome moiety of the antibody to be compensated for. Mouse cells are incubated with the MACS Comp Reagent for labeling via CD45 after which the fluorochrome-conjugated antibody in question is added. Thus, all CD45-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.

Product applications

- Efficient compensation for flow cytometric analysis of rare cells.
- Efficient compensation of tandem-fluorochrome conjugates.
- Efficient compensation for flow cytometric analysis of weakly stained cells.

1.2 Recommended reagent dilution

For labeling of mouse cells.

	MACS Comp Reagent – PE[a]	MACS Comp Reagent – APC[a]
Reagent dilution for flow cytometry	1:11	1:11
Compatible fluorochrome conjugates	PE PE-Cy®5 PE-Cy5.5 PE-Cy7 PE-Alexa Fluor® 700 PE-Alexa Fluor 750 PE-Alexa Fluor 647	APC APC-Cy7 APC-Alexa Fluor 750

a) Suitable for use with fresh or fixed cells.

▲ **Note:** Though the MACS Comp Reagent has been tested to function with a broad range of tandem fluorochrome conjugates, Miltenyi Biotec cannot guarantee that the product will work with the above listed tandem fluorochrome conjugates from all suppliers.

1.3 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 (4–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **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 mouse serum albumin, mouse 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 mouse cells, e.g. splenocytes.

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2. General protocol for MACS Comp Reagent

▲ 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.

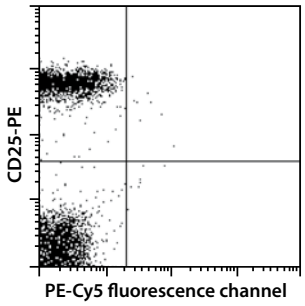
▲ Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Resuspend 5×10^5 mouse splenocytes in 100 µL of buffer.
2. Add 10 µL of MACS Comp Reagent.
3. Mix well and refrigerate for 10 minutes in the dark (4–8 °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 for at the recommended titer.
7. Mix well and refrigerate for 10 minutes in the dark (4–8 °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 5×10^5 unstained splenocytes in 500 µL of buffer to serve as an internal negative control.
11. Proceed to the 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 the fluorescence overlap (e.g. FITC fluorescence channel for PE-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.

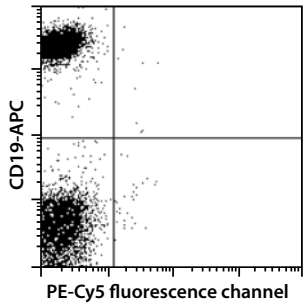
3. Examples of compensation with MACS Comp Reagent

Mouse splenocytes were labeled with MACS Comp Reagent followed by the addition of the fluorochrome-conjugated antibody to be compensated for. Unlabeled cells were added directly before measurement. Data depicting the optimal compensation of CD25-PE (# 130-091-013) (a) and CD19-APC (# 130-092-039) (b) are shown.

(a) Optimized compensation of CD25-PE using the MACS Comp Reagent-PE and mouse splenocytes.



(b) Optimized compensation of CD19-APC using the MACS Comp Reagent-APC and mouse splenocytes.



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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