MACS® All-In-One Comp Bead Kit

Order no. 130-138-867

Contents

1. Description

This product is for research use only.

Components	**5 mL MACS All-In-One Comp Beads – positive** **5 mL MACS All-In-One Comp Beads – blank**
Capacity	For 100 tests.
Product format	MACS All-In-One Comp Beads are supplied in buffer containing stabilizer.
Storage	Store protected from light at +2 to +8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

Most small molecule and protein-based fluorochromes have broad emission spectra which partially overlap with the spectra of other fluorochromes in flow cytometric analysis. This overlap should be detected and corrected for proper analysis.
The MACS All-In-One Comp Bead Kit has been developed for optimal compensation of fluorescence spillover of fluorochrome-conjugated antibodies and amine-reactive dyes. After staining with fluorochrome-conjugated antibodies and amine-reactive dyes, the MACS All-In-One Comp Beads – positive can be used for automated or manual compensation along with the MACS All-In-One Comp Beads – blank for the control of the negative population.

1.2 Applications

- Compensation of the fluorescence spillover from fluorochrome-conjugated antibodies from different species as well as amine reactive dyes for live-dead discrimination.
- Binding has been demonstrated for following species and subclasses:
  - Recombinant human (IgG1)
  - Goat (IgG)
  - Hamster (IgG, IgGκ, IgGλ, IgG1, IgG1κ, IgG1λ, IgG2, IgG2κ, IgG3κ)
  - Rat (IgG1, IgG1κ, IgG2a, IgG2aκ, IgG2aλ, IgG2b, IgG2bκ, IgG2bλ, IgG2c)
  - Mouse (IgG1, IgG1κ, IgG1λ, IgG2a, IgG2aκ, IgG2b, IgG2bκ)

1.3 Reagent and instrument requirements

- Flow cytometer, e.g., MACSQuant Analyzer 10 (# 130-096-343), MACSQuant Analyzer 16 (# 130-109-803), MACSQuant VYB (# 130-096-116), or MACSQuant X (# 130-105-100)
- MACSQuant Running Buffer (# 130-092-747)
- MACS MiniSampler Plus (# 130-105-745)
- Chill 5 Rack (# 130-092-951)
- Tubes, e.g., 5 mL round-bottom tubes
- Amine-reactive dyes, e.g., Viobility™ Fixable Dyes
- Fluorochrome-conjugated antibodies. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

2. Protocol

2.1 Reagent preparation

1. For each fluorochrome-conjugated antibody or amine-reactive dye used in the experiment label a separate 5 mL sample tube.
2. Mix the MACS All-In-One Comp Beads - positive thoroughly before use, e.g., vortex for at least 60 seconds.
3. Proceed with immunofluorescent staining (2.2).

2.2 Immunofluorescent staining

Sample setup for amine-reactive dyes

1. Add one full drop of the MACS All-In-One Comp Beads – positive to each tube.

   ▲ **Note:** One full drop is approximately 50 µL.

2. Add the appropriate amount of amine-reactive viability dye to the appropriate sample tube and ensure to dispense the dye directly to the bead suspension:

   Viobility Fixable Dyes: **5 µL.**

   ▲ **Note:** For other viability dyes the appropriate amount needs to be tested.

3. Proceed with step 5.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

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Sample setup for fluorochrome-conjugated antibodies

1. Add 50 µL of the MACSQuant Running Buffer to each sample tube.
2. Add the appropriate amount of fluorochrome-conjugated antibodies to the appropriate sample tube:

   Antibody dilution 1:10 or 1:11: **10 µL**.

   Antibody dilution 1:50: **2 µL**.

   ▲ **Note:** For other formats the appropriate amount needs to be tested.
3. Add one full drop of the MACS All-In-One Comp Beads – positive to each tube.

   ▲ **Note:** One full drop is approximately 50 µL.
4. Proceed with step 5.
5. Mix well and incubate for 30 minutes in the dark at room temperature.
6. Wash each sample by adding 1 mL of the MACSQuant Running Buffer and mix well. Centrifuge at 1000×g for 5 minutes.
7. Carefully remove the supernatant and resuspend each sample using 250 µL MACSQuant Running Buffer.
8. Mix the MACS All-In-One Comp Beads – blank thoroughly, e.g., vortex for at least 60 seconds.
9. Add one full drop of MACS All-In-One Comp Beads – blank to each tube and mix well.
10. (Optional) If using DNA dyes for live/dead discrimination instead of amine-reactive dyes prepare one additional round-bottom tube with 250 µL of MACSQuant Running Buffer and one drop of the MACS All-In-One Comp Beads – positive and mix well. Do not add any staining. Only spectral overlap into the viability channel is compensated.
11. Proceed with compensation using the Express Mode CompensationMultiColor (2.3).

2.3 Compensation using the Express Mode CompensationMultiColor

▲ Refer to the respective user manual for instructions on how to use the MACSQuant Instrument.

▲ The MACSQuant Instrument must be calibrated beforehand, refer to the short instructions "Photomultiplier tube (PMT) calibration" for detailed information.

1. Change forward scatter (FSC) and side scatter (SSC) instrument settings from lin to **log3** and reduce the SSC voltage by 50 V.
2. Click on the **Experiment** tab and choose **Chill 5 tube rack.** Place the sample tubes into the rack.
3. Select the choosen rack positions and group them by clicking the **Group** button in the rack template.
4. Choose **Express** mode in the **Settings** tab.
5. Select **Setup** from the **Type** drop-down menu.
6. Select **CompensationMultiColor** from the **Mode** drop-down menu.
7. Select a position in the rack window. Choose the appropriate color for each rack position from the **SampleID** drop-down menu. Refer to table 1 for correct assignment if using viability dyes for live/dead discrimination. If using DNA dyes for live/dead discrimination, only spectral overlap into the viability channel is compensated.
8. Click the **Run** button to start the acquisition. Follow the instructions on the screen.

MACSQuantify Software version	Propidium iodide (PI)	7-AAD	Amine reactive dyes
≥ 3.0.1	Select the respective viability channel from the lower half of the drop-down menu.		Select the respective channel for the used dye. Do not select one of the viability channels from the lower half of the dropdown menu.
2.13.2 2.13.3	Select **PI**	Select **7-AAD**	Select the respective channel for the used dye. Do not select other viability dye.
≤ 2.13.1	Select **PI**	n.a.*	Select the respective channel for the used dye.

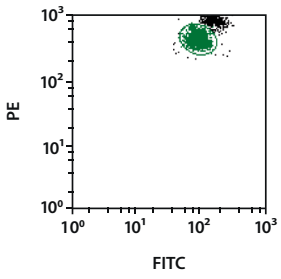
*When working with MACSQuant Analyzer 10, select **PI**.
Automatic 7-AAD compensation with MACS All-In-One Comp Beads – blank is not available for MACSQuant Analyzer 16.

Table 1: Channel assignment from the **SampleID** drop-down menu for DNA staining and amine-reactive dyes for live/dead discrimination.

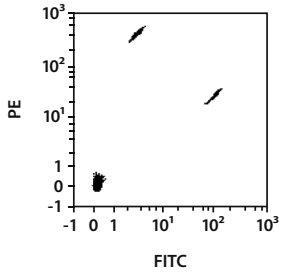
3. Example of a compensation using the MACS All-In-One Comp Bead Kit

The MACS All-In-One Comp Beads were stained with antibodies conjugated to FITC and PE and after defining the region of interest (A) analyzed by flow cytometry using the Express Mode CompensationMultiColor on a MACSQuant Analyzer 16. MACS All-In-One Comp Beads are shown during (B; uncompensated) and after (C; compensated) running the Express Mode CompensationMultiColor.

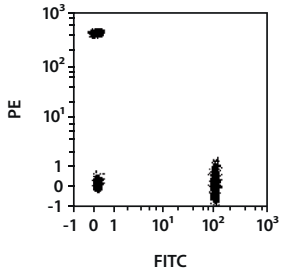
A)



B)



C)



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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