Miltenyi Biotec

MACS® Comp Bead Kit, anti-rat Igκ

Order no. 130-107-755

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	**5 mL MACS Comp Beads – anti-rat Igκ** **5 mL MACS Comp Beads – blank**
Capacity	For 100 tests.
Product format	MACS Comp Beads 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

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 Comp Bead Kit, anti-rat Igκ has been developed for optimal compensation of fluorescence spillover of fluorochrome-conjugated antibodies. After staining with any rat Igκ fluorochrome-conjugated antibodies, the MACS Comp Beads – anti-rat Igκ can be used for automated or manual compensation along with the MACS Comp Beads – blank for the control of the negative population.

1.2 Applications

- Compensation of fluorescence spillover of rat Igκ fluorochrome-conjugated antibodies.

1.3 Reagent and instrument requirements

- MACSQuant® Analyzer, MACSQuant Analyzer 10 (# 130-096-343), or MACSQuant VYB (# 130-096-116)
- MACSQuant Running Buffer (# 130-092-747)
- MACS MiniSampler Plus (# 130-105-745)
- Chill 5 Rack (# 130-092-951)
- MACSQuant Calibration Beads (# 130-093-607)
- Rat Igκ fluorochrome-conjugated antibodies. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

2. Protocol

2.1 Immunofluorescent staining

1. Mix the MACS Comp Beads – anti-rat Igκ and MACS Comp Beads – blank well before use.
2. For each fluorochrome-conjugated rat Igκ antibody to be used in the experiment label a separate 5 mL sample tube.
3. Add 100 µL of the MACSQuant Running Buffer to each sample tube.
4. Add the appropriate amount of rat Igκ fluorochrome-conjugated antibodies to the appropriate sample tube:

   Antibody dilution 1:10 or 1:11: **20 µL**.

   Antibody dilution 1:50: **4 µL**.

   ▲ **Note:** For other formats use 1 µg/mL.
5. Add one full drop of the MACS Comp Beads – anti-rat Igκ and one full drop of the MACS Comp Beads – blank to each tube.

   ▲ **Note:** One full drop is approximately 50 µL.
6. Mix well and incubate for 5–10 minutes in the dark at room temperature (19–25 °C). Shake sample during incubation.
7. Dilute each sample by adding 1 mL of the MACSQuant Running Buffer and mix well.
8. (Optional) If no fluorochrome for channel B3 on the MACSQuant Analyzer or B2 on the MACSQuant VYB is used, add 500 µL of MACSQuant Running Buffer and one drop of the MACS Comp Beads – blank to a separate tube and mix well. This sample will be used to set the compensation values for propidium iodide (PI).

   ▲ **Note:** Fluorochromes for channel B3 or B2 are, for example, PerCP, PE-Cy® 5, or PerCP-Vio® 700.
9. Proceed with calibration of the MACSQuant Instrument (2.2).

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2.2 Calibration of the MACSQuant Instrument

▲ Refer to the respective user manual for instructions on how to use the MACSQuant Analyzer, MACSQuant Analyzer 10, or MACSQuant VYB.

1. Login and prime the MACSQuant Instrument. Check that the system status bar indicates 'Ready' (green).
2. Ensure that the single tube holder is correctly attached to the instrument.
3. Resuspend the MACSQuant Calibration Beads by vortexing.
4. Click on the Barcode reader icon. Position the barcode label of the MACSQuant Calibration Beads vial in front of the barcode reader.

   ▲ **Note:** A dialog box will appear that instructs the user to add one drop of calibration beads to a single tube.
5. Place the tube containing one drop of Calibration Beads into the single tube holder and click on **run**. The instrument will then proceed to automatically dilute and mix the bead suspension before performing calibration.

   ▲ **Note:** The system status bar will indicate 'processing sample' (blue) until completion of the task.
6. Following successful calibration the status bar will report 'MACSQuant ready; Calibration ok'; this is visually represented by a green status.

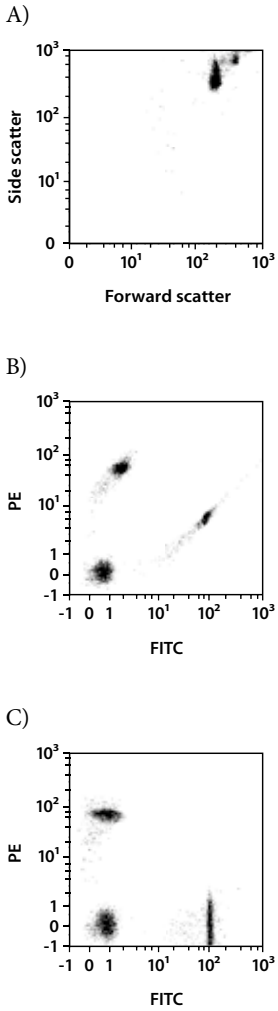
   ▲ **Note:** The gain and trigger settings are now preset for running experiments and can be viewed under the channel tab in the **Instrument Settings**.
7. Proceed to compensation (2.3).

2.3 Compensation

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 **Group** in the rack template.
4. Choose **Express** mode in the **Settings** tab. Choose type **Setup** and mode **CompensationMultiColor** from the dropdown menues.
5. Choose the appropriate color for each rack position under **SampleID**.
6. (Optional) If using MACS Comp Beads – blank for PI compensation, select **PI** in the **SampleID** for this tube.
7. Click on run and follow the given instructions by the MACSQuant Instrument.

3. Example of a compensation using the MACS Comp Bead Kit

The MACS Comp Beads – anti-rat Igκ were labeled with antibodies conjugated to FITC and PE and analyzed. Gating was performed on single bead events (A). MACS Comp Beads are shown before (B) and after (C) compensation using the Compensation Multicolor program with the MACSQuant Analyzer.



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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