MACS® Comp Bead Kit, anti-REA

Order no. 130-104-693

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1. Description

This product is for research use only.

Components	**5 mL MACS Comp Beads – anti-REA** **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 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 Comp Bead Kit, anti-REA has been developed for optimal compensation of fluorescence spillover of fluorochrome-conjugated REAfinity™ Antibodies. After staining with fluorochrome conjugated REAfinity Antibodies, the MACS Comp Beads – anti- REA 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 the fluorescence spillover from fluorochrome-conjugated REAfinity® Antibodies.

2. Protocol for usage on a MACSQuant Analyzer

2.1 Reagent and instrument requirements

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

2.2 Immunofluorescent staining

1. Mix the MACS Comp Beads – anti-REA and MACS Comp Beads – blank well before use.
2. For each fluorochrome-conjugated REAfinity Antibody 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 fluorochrome-conjugated REAfinity Antibodies to the appropriate sample tube. For an antibody dilution 1:10 or 1:11 use 20 µL. For an antibody dilution 1:50 use 4 µL.

   ▲ **Note:** For other formats use 1 µg/mL.
5. Add one full drop of the MACS Comp Beads – anti-REA 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. Shake sample during incubation.
7. Dilute each sample by adding 1 mL of the MACSQuant Running Buffer and mix well.
8. (Optional) If using DNA- or amine-reactive dyes for live/dead cell discrimination, prepare one additional round-bottom tube with 500 µL of MACSQuant Running Buffer and one drop of the MACS Comp Beads - blank and mix well.

   ▲ **Note:** Do not add any staining reagent. Only spectral overlap into the viability channel is compensated.
9. Proceed with compensation of the MACSQuant Analyzer Instrument.

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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2.3 Compensation

▲ Before starting the compensation, ensure a proper calibration of the device. Refer to the respective user manual for instructions on how to calibrate the MACSQuant Analyzer Instruments.

▲ Refer to the latest MACSQuant Instrument short instructions - Photomultiplier tube (PMT) calibration (3.0)

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** dropdown menu.
7. Choose the appropriate color for each rack position under **SampleID**.
8. (Optional) If using a viability dye select the respective channel (e.g. PI, 7-AAD or other viability dye) from the **SampleID** drop-down menu.
9. Click the **Run** button and follow the given instructions by the MACSQuant Analyzer Instrument.

3. Protocol for usage on a MACSQuant Tyto® Instrument

3.1 Reagent and instrument requirements

- Flow cytometer, e.g., MACSQuant Tyto Cell Sorter (# 130-103-931) and MACSQuant Tyto Lux (# 130-133-903) with MACSQuantify™ Tyto Software version 3.2 or higher
- MACSQuant Running Buffer (# 130-092-747)
- MACSQuant Tyto Cartridges (# 130-104-791, # 130-106-088), MACS GMP Tyto Cartridge (# 170-076-011), or MACSQuant Tyto Cartridges HS (# 130-121-549, # 130-121-551), or MACS GMP Tyto Cartridge HS" (# 170-084-001)
- Fluorochrome-conjugated REAfinity Antibodies. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

3.2 Immunofluorescent staining

1. Label a separate sample tube for each fluorochrome-conjugated antibody used in the experiment. These are the single stain controls.
2. Label one additional sample tube for the blank beads.
3. Add 150 µL of the MACSQuant Running Buffer to each single stain control sample tube.
4. Vortex the MACS Comp Beads and the MACS Comp Beads – blank thoroughly before use.
5. Add one full drop of MACS Comp Beads per single stain control.
6. Add the appropriate amount of fluorochrome-conjugated antibodies to the appropriate sample tube. For an antibody dilution of 1:50 use 4 µL antibody.
7. Mix well and incubate for 5 to 10 minutes in the dark at room temperature. Shake sample during incubation.
8 Prepare the blank sample during incubation. Add 250 µL of the MACSQuant Running Buffer to the blank sample tube and add one full drop of the MACS Comp Beads – blank. The blank sample does not require washing.
9. To wash the single stain controls, add 1 mL of MACSQuant Running Buffer to each single stain control sample and mix well.
10. Centrifuge the single stain control sample tubes at 300×g for 5 minutes at room temperature.
11. Carefully aspirate or decant the supernatant.
12. Resuspend each pellet in 300 µL MACSQuant Running Buffer.
13. Ensure that the MACSQuant Tyto Instrument is calibrated and the workspace is set up. See protocol MACSQuant Tyto Instrument short instructions - Compensation, steps 1–5.
14. Load the single stain control samples and the blank sample into the cartridge. Load equal volumes of the single stain control samples.

    ▲ **Note:** It is recommended to at least provide 500 µL of compensation sample in the cartridge. For example: For 2 color compensation panel, total volume of compensation sample consists of 2×167 µL from the two single stain samples plus 1×167 µL from blank sample = ca. 500 µL of total compensation sample.
15. Proceed immediately with the automated compensation. See protocol MACSQuant Tyto Instrument short instructions - Compensation, steps 6–10.

    ▲ **Note:** Do not store the mixed compensation control sample.

3.3 Compensation

▲ Before mixing the compensation single stain controls together in the cartridge, the following prerequisites should be met: the instrument should be calibrated and the gains should be set-up appropriately (i.e. discrimination of populations possible, target dye brightest in target channel). Refer to the MACSQuantify™ Tyto® Software 3.2 user manual for instructions on how to calibrate the MACSQuant Tyto Instruments.

▲ Refer to the latest MACSQuant Tyto Instrument short instructions - Photomultiplier tube (PMT) calibration.

1. Ensure that the MACSQuant Tyto Instrument is calibrated.
2. Go to **File > New workspace** to open an empty workspace.
3. (Optional) Open an existing workspace containing the correct PMT channel values. Refer to the MACSQuantify Tyto Software 3.2 user manual, chapter 7.
4. Adjust all PMT channels. For details refer to chapter "Setting fluorescence channel gains" in the MACSQuantify Tyto Software 3.2 user manual.
5. Adjust the sample volume in the Experiment tab according to the provided volume in the cartridge.
6. Scan and insert the cartridge containing the compensation control samples.

    ▲ **Note:** It is recommended to at least provide 500 µL of compensation sample in the cartridge. For example: For 2 color compensation panel, total volume of compensation sample consists of 2×167 µL from the two single stain samples plus 1×167 µL from blank sample = ca. 500 µL of total compensation sample.

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7. Follow the instructions in the dialog box.
8. When prompted, adjust the region around the scatter population in the BSV-H/BSB-H plot that defines the cells or beads used for compensation. If working with cells, make sure to exclude the debris. Include only cells which are relevant for the sorting strategy in the region of interest (ROI).
9. When compensation is completed, a dialog box opens to apply the calculated spillover matrix to your workspace. Click **Yes**.
10. Another dialog box opens. Click **Yes** to save the workspace. This will save the spillover matrix to your workspace.

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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