MACSQuant® Advanced Calibration Beads

Order no. 130-134-208

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1. Description

This product is for research use only.

Components	1×4 mL MACSQuant Advanced Calibration Beads
Capacity	For up to 100 tests.
Product format	MACSQuant Advanced Calibration Beads are supplied in phosphate-buffered saline containing 0.05% Tween® 20 and 0.05% sodium azide.
Storage	Store bottle protected from light at +2 to +8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

The MACSQuant Advanced Calibration Beads are designed for routine calibration of a MACSQuant Flow Cytometer.
The MACSQuant Advanced Calibration Beads are 2.0, 2.6, 3.2, and 5.8 µm in size and enable the adjustment of voltage settings. The 2 µm and the 2.6 µm particles are stained in dim and bright intensities, respectively, with multiple fluorescent dyes in the range of 400 nm to 650 nm for excitation. They are emitting in all fluorescent channels. The 3.2 µm particles are single-stained with fluorescent dyes yielding medium bright signals in target channels and allow for target median fluorescent intensity (MFI) calibration. The 5.8 µm particles are unstained.

1.2 Applications

- Calibration of scatter and fluorescence settings of the MACSQuant Analyzer with MACSQuantify™ Software version 3.1 or higher

1.3 Reagent and instrument requirements

- MACSQuant 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)
- Tubes, e.g., 5 mL round-bottom tubes

2. Protocol

▲ For a detailed description regarding the use of the MACSQuant Flow Cytometer refer to the appropriate section of the user manuals. The following guidelines are for performing an automated photomultiplier tube (PMT) calibration. Refer to the MACSQuantify Software 3.1 user manual for details concerning manual calibration.

▲ Use MACSQuantify Software version 3.1 or higher.

1. Login and prime the MACSQuant Flow Cytometer. Wait at least 30 minutes to warm-up the optical bench. Check that the system status bar indicates 'Ready' (green).
2. Ensure that the single tube rack is correctly attached to the instrument.
3. Resuspend the MACSQuant Advanced Calibration Beads by vortexing for 10 seconds.
4. Click on the Barcode reader icon. Place the bottle of the MACSQuant Advanced Calibration Beads in the right recess of the single tube rack with the multi-region (left) barcode facing the instrument.

   ▲ **Note:** A dialog box will appear that instructs the user to add one drop of calibration beads to a single tube.
5. Add one drop of MACSQuant Advanced Calibration Beads into an empty tube.
6. Place the tube into the single tube rack 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.
7. 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**.

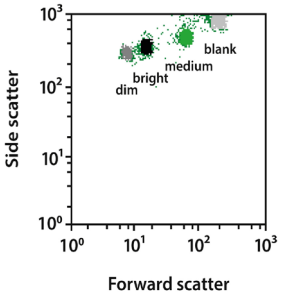
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3. Example of acquisition of MACSQuant Advanced Calibration Beads on a MACSQuant Analyzer

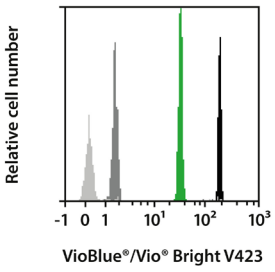
Forward scatter-area (FSC-A) versus side scatter-area (SSC-A)
The forward versus side scatter plot (A) can be used to distinguish the different sizes of the MACSQuant Advanced Calibration Beads. Blank particles with 5.8 µm in size are displayed in light grey, the 2.0 µm dim particles in medium grey and the 2.6 µm bright particles in black. The 3.2 µm particles being a mixture of single-stained medium bright particles are displayed in green.

A)

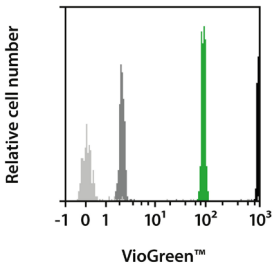


Fluorescence histograms displaying the different fluorescent properties of the 2.0, 2.6, 3.2, and 5.8 µm size particles
Histograms show the fluorescence intensity of the MACSQuant Advanced Calibration Beads after excitation with the 405 nm (B–F), 488 nm (G–L), and 640 nm (M–O) lasers of the MACSQuant Analyzer 16. Examples of the corresponding fluorochrome for each fluorescence channel are indicated in the axis legend. As in plot A), blank particles with 5.8 µm in size are displayed in light grey, the 2.0 µm dim particles in medium grey, and the 2.6 µm bright particles in black. The 3.2 µm single-stained medium bright particles are displayed in green.

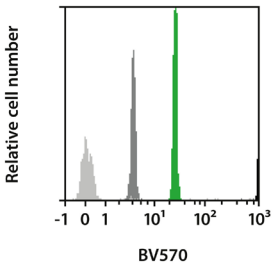
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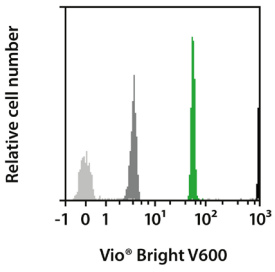
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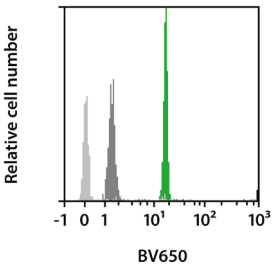
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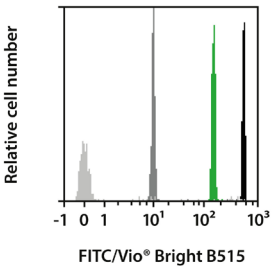
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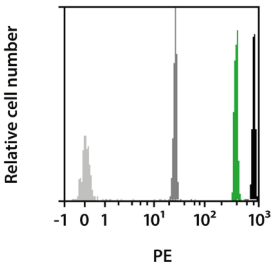
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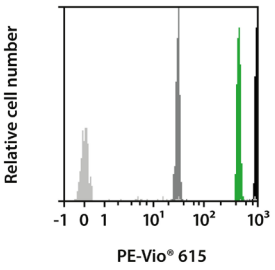
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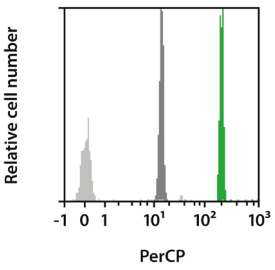
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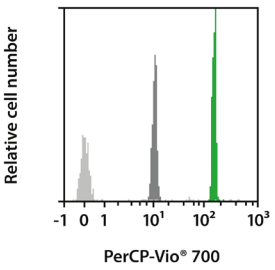
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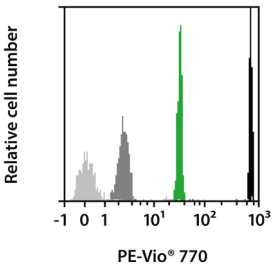
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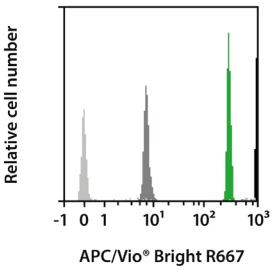
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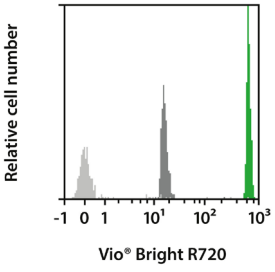
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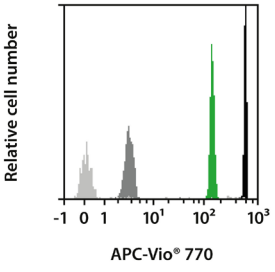
M)



N)



O)



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