Miltenyi Biotec

MACSQuant® Tyto® Calibration Beads

Order no. 130-122-730

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1. Description

This product is for research use only.

Components	1×4 mL MACSQuant Tyto Calibration Beads
Capacity	For at least 25 tests.
Product format	MACSQuant Tyto Calibration Beads are supplied in phosphate-buffered saline containing 0.05% Tween® 20 and 0.05% sodium azide.
Storage	Store the vial 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 Tyto Calibration Beads are designed for routine calibration of the MACSQuant Tyto Instruments.
The MACSQuant Tyto 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 in intermediate 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 fluorescence settings of the MACSQuant Tyto Instruments with MACSQuantify Tyto Software version 3.0 or higher

1.3 Reagent and instrument requirements

- MACSQuant Tyto Cell Sorter (# 130-103-931) or MACSQuant Tyto Lux Cell Sorter (# 130-133-903) with MACSQuantify™ Tyto Software version 3.0 or higher
- MACSQuant Running Buffer (# 130-092-747) or MACSQuant Tyto Running Buffer (# 130-107-207)
- MACSQuant Tyto Cartridges (# 130-104-791, # 130-106-088), MACS GMP Tyto Cartridge (# 170-076-011), MACS GMP Tyto Cartridge HS (# 170-084-001), or MACSQuant Tyto Cartridges HS (# 130-121-549, # 130-121-551)

2. Protocol

▲ Cartridge priming is mandatory before loading the cartridge with MACSQuant Tyto Calibration Beads into the MACSQuant Tyto Instrument. For more information refer to the respective data sheet or the short instructions on cartridge priming and sample loading.

▲ For a detailed description regarding the use of the MACSQuant Tyto Instrument refer to the appropriate section of the user manuals. The following guidelines are for performing an automated photomultiplier tube (PMT) calibration.

▲ Use MACSQuantify Tyto Software version 3.0 or higher.

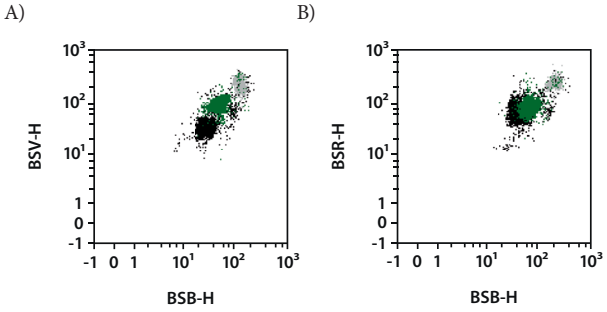
1. Switch on the MACSQuant Tyto Instrument. Wait at least 30 minutes to warm-up the optical bench before performing the automated PMT calibration.
2. Vortex the MACSQuant Tyto Calibration Beads for 10 seconds to break up aggregates.
3. Prepare a cartridge containing three drops MACSQuant Tyto Calibration Beads in 1.5 mL MACSQuant Running Buffer or MACSQuant Tyto Running Buffer.
4. Scan and insert the cartridge.
5. Scan the barcode of the MACSQuant Tyto Calibration Beads.
6. Click **Start** on the Daily calibration screen to start the calibration. The gain for each channel is automatically adjusted during calibration.
7. The calibration results for each channel are presented as dot plots, histograms, and as a tabulated summary.
8. Successful calibration is indicated for each channel by the column **Info** giving the status **passed**. Furthermore, the table header of the calibration analysis page shows **Calibration: passed** and the instrument status bar reports **calibration ok**. Gains and threshold will be automatically saved as a default setting in the workspace.

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

3. Example of acquisition of MACSQuant Tyto Calibration Beads on the MACSQuant Tyto Cell Sorter

Backscatter blue (BSB) versus backscatter violet (BSV) (A) or backscatter red (BSR) (B)

The dot plots display height of backscatter across the three lasers (blue, violet, and red) and depict the different sizes of the MACSQuant Tyto Calibration Beads. These plots are further used to detect fluorescent intensities (C–J) during the automated calibration process.

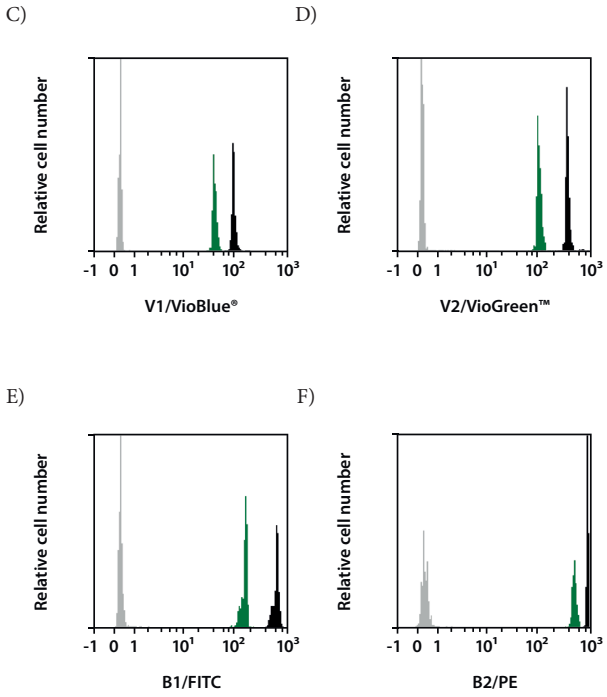


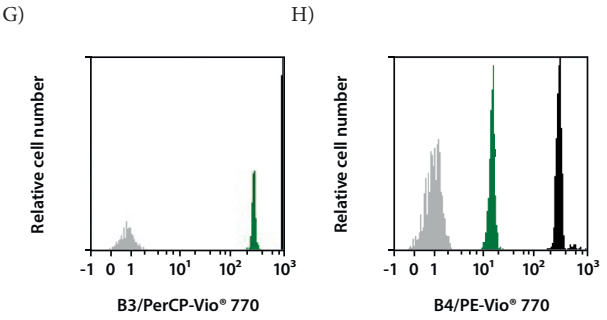
Fluorescence of 2.0, 2.6, 3.2, and 5.8 µm size particles

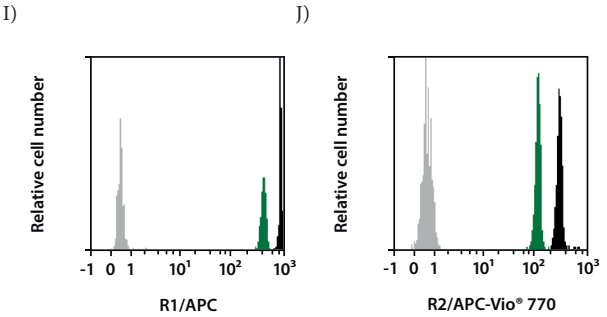
Histograms show the fluorescence intensities of the multi-color calibration beads after excitation with the 405 nm (G, H), 488 nm (C–F), and 635 nm (I, J) lasers of the MACSQuant Tyto Cell Sorter. An example of the corresponding fluorochrome in the respective fluorescence channel is indicated in the axis legend.

The unstained 5.8 µm particles were used as blanks (light gray; C–J) and the 2.6 µm particles as brights (black; C–J). The 2 µm dim particles were excluded as they are not used for the calculation of the automated PMT calibration (data not shown).

The 3.2 µm particles were detected either in all violet channels (green; C–D), all blue channels (green; E–H), or all red channels (green; I–J).







4. Example of acquisition of MACSQuant Tyto Calibration Beads on the MACSQuant Tyto Lux Cell Sorter

The MACSQuant Tyto Lux Cell Sorter is equipped with an additional yellow laser which is in collinear configuration with the blue laser and comes with 12 channels: 4 channels on the violet laser (V1–V4), 1 channel on blue laser (B1), 4 channels on the blue yellow collinear lasers (B/Y2–B/Y5), and 3 channels on the red laser (R1–R3).

Shown here are only the histograms of the new channels coming with the MACSQuant Tyto Lux Cell Sorter. The other channels which are not shown here are exactly the same shown in chapter 3 for MACSQuant Tyto Cell Sorter.

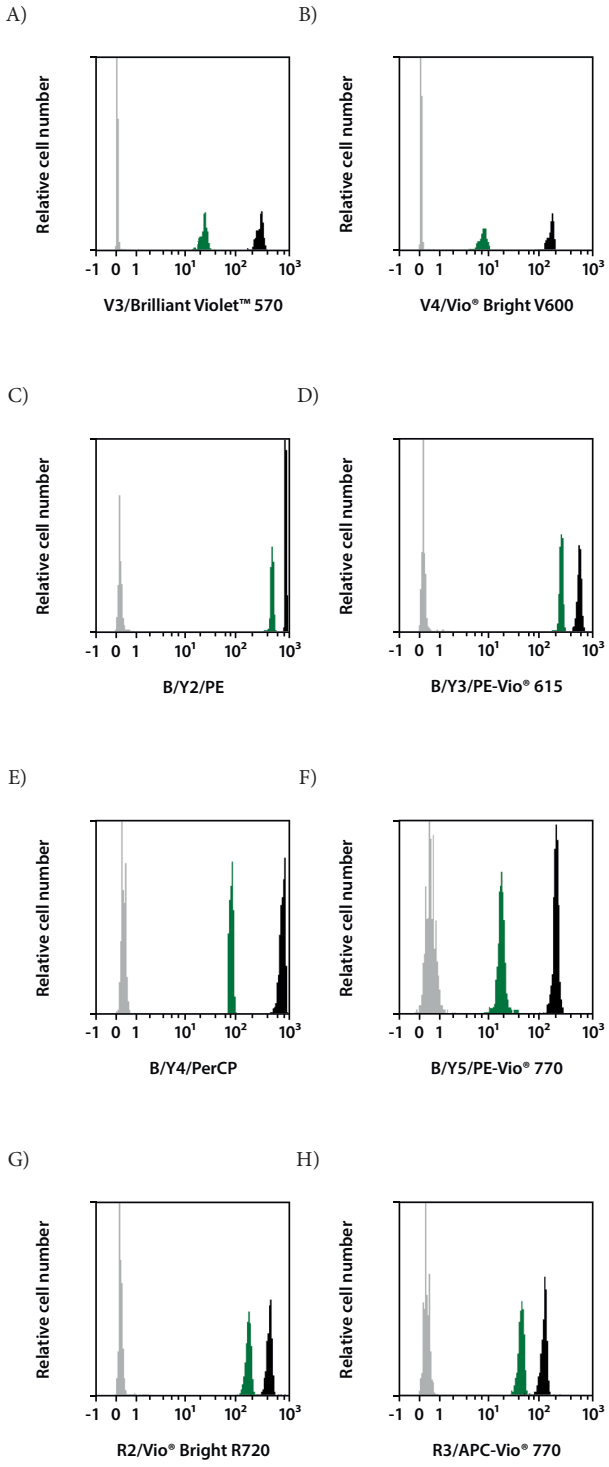
Histograms show the fluorescence intensities of the multi-color calibration beads after excitation with the 405 nm, 488 nm/552 nm, and 635 nm lasers of the MACSQuant Tyto Lux Cell Sorter. An example of the corresponding fluorochrome in the respective fluorescence channel is indicated in the axis legend.

The unstained 5.8 µm particles were used as blanks (light gray;) and the 2.6 µm particles as brights (black;). The 2 µm dim particles were excluded as they are not used for the calculation of the automated PMT calibration (data not shown).

The 3.2 µm particles were detected either in all violet channels (green, A–B), all blue and yellow channels (green, C–F), or all red channels (green, G–H).

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