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## 1. Description

<b>Components</b>	MACSQuant™ Calibration Beads.
<b>Capacity</b>	100 tests.
<b>Product format</b>	MACSQuant Calibration Beads are supplied in aqueous solution containing 0.02% Tween® 20 and 0.05% sodium azide.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

### 1.1 Background information

The MACSQuant Calibration Beads are designed for routine calibration of the MACSQuant Analyzer. These calibration particles are 2 and 3 µm in size and enable the adjustment of the voltage settings. In addition, the 3 µm size particles are stained with different fluorescent dyes, whose emission spectra are compatible with many commonly used fluorochromes for flow cytometry. The MACSQuant Calibration Beads are excited by light between 400 nm and 650 nm to yield emission in all fluorescent channels. This enables the successful calibration of the laser settings.

### 1.2 Applications

- Calibration of the scatter and fluorescence settings of the MACSQuant Analyzer.

### 1.3 Reagent requirements

- MACSQuant Running Buffer (# 130-092-747)

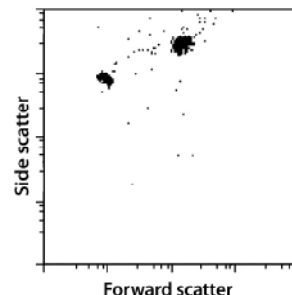
## 2. Protocol

▲ For a detailed description regarding the use of the MACSQuant Analyzer refer to the appropriate section of the user manual. The following guidelines are for performing an automated calibration only. Refer to the MACSQuant Analyzer user manual for details concerning manual calibration.

1. Login and prime the MACSQuant Analyzer. 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**.

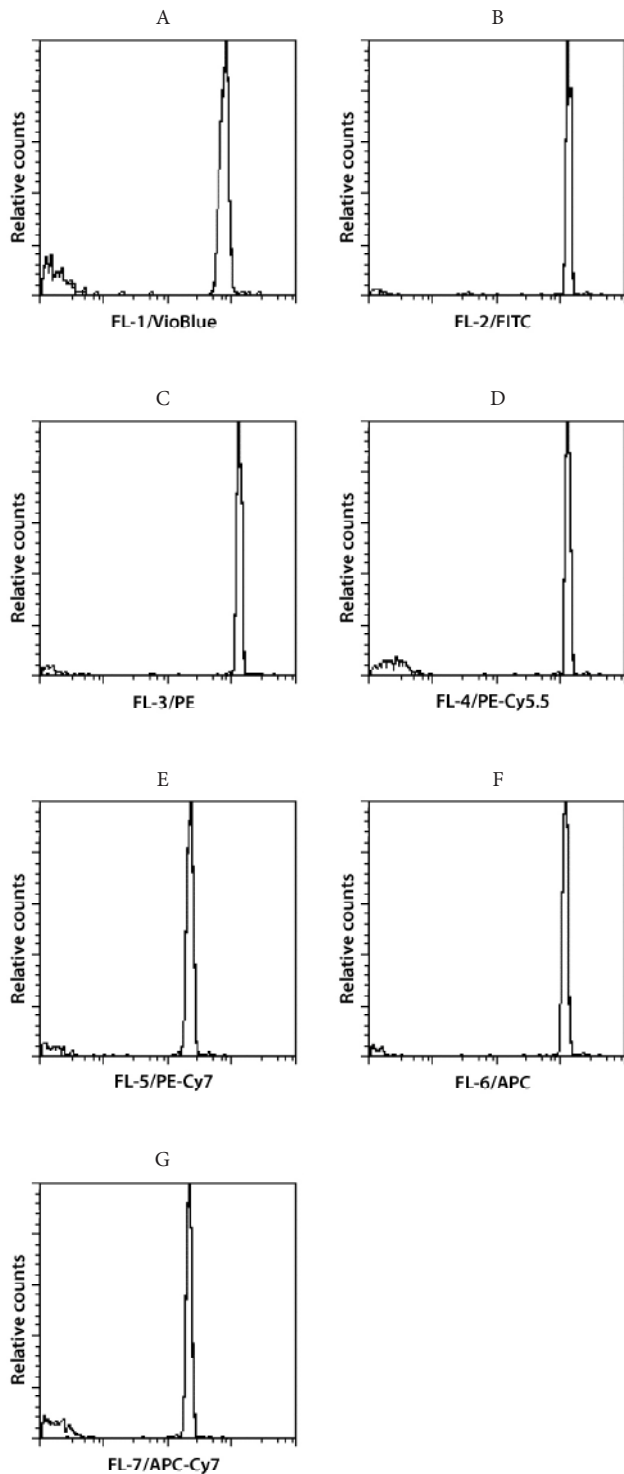
## 3. Example of acquisition of MACSQuant™ Calibration Beads on a MACSQuant™ Analyzer

- (a) Forward scatter (FSC) versus side scatter (SSC)



## (b) Fluorescence

Histograms show the fluorescence intensity of the Calibration Beads after excitation with the 405 nm (A), 488 nm (B, C, D, E), and 635 nm (F, G) lasers. Examples of the corresponding fluorochrome in the respective fluorescence channel are indicated in the axis legend.



All protocols and data sheets are available at [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

**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.

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