



Miltenyi Biotec



MACSQuant® Instrument short instructions

Photomultiplier tube (PMT) calibration

Before using the instrument for the first time, read the MACSQuant Instrument user manual and MACSQuantify Software user manual.

Introduction

The reproducibility and stability of the fluorescence signal over time is of vital importance. In order to ensure a stable measurement that is independent of time, the instrument needs to be calibrated. Calibrate the MACSQuant Instrument using the MACSQuant Calibration Beads. A daily calibration compensates for potential differences in laser performance or PMT sensitivity by adjusting the voltages accordingly. MACSQuant Calibration Beads contain blank beads and brightly fluorescent beads, which are detected in all channels. The beads serve as a reference to establish pre-determined, lot-specific median fluorescence intensity (MFI) values for each channel. This is achieved by adjusting the voltages on each of the PMTs for the appropriate channel during the automated PMT calibration process.

It is recommended to calibrate the instrument on every day the instrument is used.

Materials required

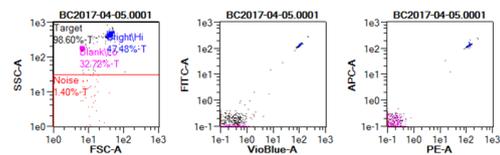
- MACSQuant Calibration Beads (# 130-093-607)
- 12x75 mm (5 mL) tube or microcentrifuge tube
- MACSQuant Running Buffer (# 130-092-747)

Automated PMT calibration

- 1 Ensure that the MACSQuant Instrument is primed and has been in acquisition mode for at least 30 minutes.
- 2 Go to the **Experiment** tab.
- 3 Select **Single tube rack** from the **Rack** drop-down menu.
- 4 Vortex the MACSQuant Calibration Beads for 10 seconds to break up aggregates.
- 5 Click the **Barcode** button on the toolbar to activate the 2D barcode reader.
- 6 Scan the 2D code printed on the vial label of the MACSQuant Calibration Beads and follow the dialog box instructions.
- 7 Dispense one drop into an empty tube.
- 8 Place the tube in the Single tube rack.
- 9 Click **OK** to start the calibration. The calibration beads are automatically diluted to a total volume of 300 µL. The voltage for each channel is automatically adjusted during calibration.

- 10 The calibration results for each channel are presented as dot plots, histograms, and as a tabulated summary on several pages. Click the **Next window** or **Previous window** button to switch between the analysis window pages.
- 11 Successful calibration is indicated for each channel by a green check mark in the table column **State**. When the process is successfully completed, the instrument status bar reports **Acquisition Mode: Calibration OK**.
- 12 Voltages and trigger will be automatically saved as a default instrument setting in **Public**.

Voltages and trigger of the PMT calibration are loaded as an active instrument setting when a new workspace is generated via **File > New workspace**.



Calibration [2017-04-05]: Passed

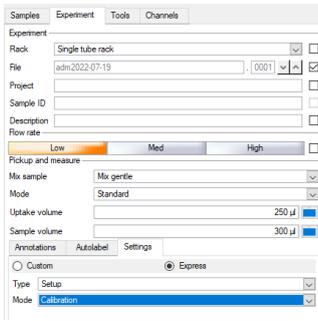
EM Packet Version: 28.2.16134 Description: Calibration Beads Lot No.: 5161017221

Channel	Gain	Diff.	SI	State	info
FSC	414	2	74.57	✓	passed!
SSC	566	2	23.03	✓	passed!
V1	494	-4	154.55	✓	passed!
V2	552	-6	903.24	✓	passed!
B1	496	0	864.15	✓	passed!
B2	568	-2	557.53	✓	passed!
B3	524	0	672.52	✓	passed!
B4	656	2	108.62	✓	passed!
R1	538	0	602.42	✓	passed!
R2	612	-2	32.88	✓	passed!

Trigger	Value	Diff.	Noise	State	info
FSC	15.94	0.15	1.08	✓	passed!

Manual PMT calibration

- 1 Ensure that the MACSQuant Instrument is primed and has been in acquisition mode for at least 30 minutes.
- 2 Go to the **Experiment** tab.
- 3 Select **Single tube rack** from the **Rack** drop-down menu.
- 4 Vortex the MACSQuant Calibration Beads for 10 seconds to break up aggregates.
- 5 Dispense one drop into an empty tube.
- 6 Add 270µL MACSQuant Running Buffer.
- 7 Place the tube in the Single tube rack.
- 8 Select the **Settings** tab and select **Express**.
- 9 Go to the **Type** drop-down menu and select **Setup**.
- 10 Go to the **Mode** drop-down menu and select **Calibration**.



- 11 Click the  **Run** button in the instrument status bar. The voltage for each channel is automatically adjusted during calibration.
- 12 The calibration results for each channel are presented as dot plots, histograms, and as a tabulated summary on several pages. Click the  **Next window** or **Previous window** button to switch between analysis window pages.
- 13 Successful calibration for each channel is indicated by a green check mark. When the process is successfully completed, the instrument status bar reports **Acquisition Mode: Calibration OK**.
- 14 Voltages and trigger will be automatically saved as a default instrument setting.

Voltages and trigger of the PMT calibration is loaded as an active instrument setting when a new workspace is generated via **File > New workspace**.

Troubleshooting PMT calibration

Calibration failed

A failed calibration may have the following causes:

1. High CV of fluorescence channels
 - Confirm that the optical bench warmed up for at least 30 minutes.
 - Run several samples of 1% hypochlorite solution. Refer to the short instructions **Maintenance**. Repeat the PMT calibration.
 - Laser alignment may have drifted. Contact Miltenyi Biotec Technical Support or initiate a MACSQuant Live Support session for assistance. If possible provide the data file of the failed calibration and the log file of the day of calibration.
2. High noise
 - Run a **Clean program**. Repeat the PMT calibration.
 - Release air from pallfilter when the instrument is in acquisition mode. Repeat the PMT calibration.
 - Check if the tubings from the running buffer bottle are tightly connected.

Calibration incomplete

An incomplete calibration indicates that not enough beads were measured during calibration.

- Make sure to vortex the calibration beads thoroughly before use.
- Repeat calibration after adding more calibration beads to the tube.
- Run a **Clean program**. Repeat the PMT calibration.
- Check needle arm calibration. For more information, refer to the MACSQuant Instrument user manual.
- Contact Miltenyi Biotec Technical Support or initiate a MACSQuant Live Support session for assistance. If possible, provide the data file of the failed calibration and the log file of the day of calibration.

Are you in need of additional assistance?

Miltenyi Biotec provides products and services worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.