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

Calibrating MACSQuant Instruments using MACSQuant Calibration Beads (# 130-093-607) is vital for maintaining the reproducibility and stability of fluorescence measurements over time. A daily calibration corrects potential differences in laser performance or PMT sensitivity. MACSQuant Calibration Beads contain blank beads, and brightly stained fluorescent beads that are detected in all channels. The beads serve as a reference to establish predefined, lot-specific median fluorescence intensity values for each channel. This is achieved by adjusting the voltages on each PMT during the automated PMT calibration process.

It is recommended to calibrate the instrument at the beginning of every day the instrument is used.

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 in the toolbar to activate the 2D barcode reader.
6. Scan the 2D code printed on the vial label of the MACSQuant Calibration Beads. A window opens.
7. Select **Yes** to continue with calibration.
8. Follow the instructions in the next window.
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.

Do not change gates or plot axes during calibration to ensure successful calibration.

10. The calibration results for each channel are presented as dot plots, histograms, and as a tabulated summary (**Figure 1**). Click the **Next** or **Previous** button to switch between the analysis windows. Successful calibration is indicated for each channel by a green check mark for the stain index **SI** and the coefficient of variation **CV**. Furthermore, the table header of the calibration analysis page shows **Passed** and the instrument status bar reports **Acquisition Mode: Calibration OK**. Refer to **Table 1** for details about the shown parameter.

    The PMT calibration is automatically saved as default instrument setting in **Public**.

Voltages and trigger of the PMT calibration are loaded as default instrument setting when a new workspace is generated via **File > New workspace** and after every log in.

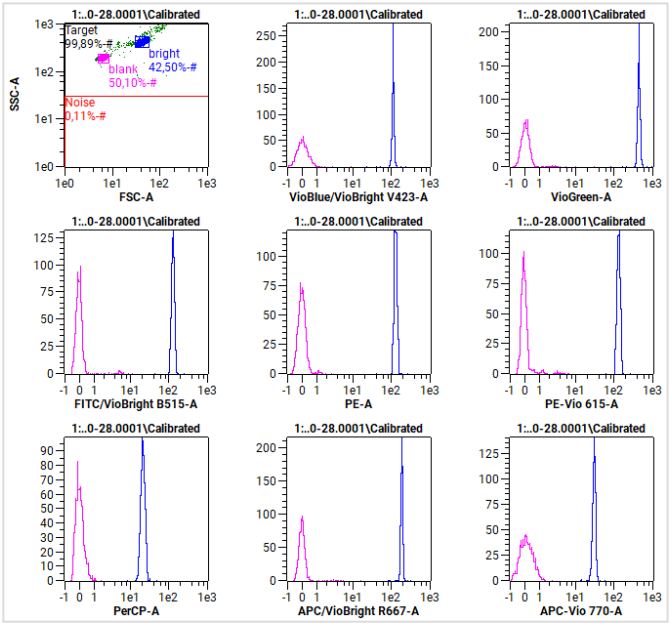


Figure 1: Calibration results of a MACSQuant Analyzer 10. Results look slightly different for other MACSQuant Instrument types.

Parameter	Explanation
V	voltage on PMT
Δ(V)	difference from last successful calibration
Δ Init(V)	difference from last successful initial calibration
SI	stain index
CV	coefficient of variation based on MFI
Δ(T)	difference in temperature from the start of calibration to the end of calibration
range	acceptable range of temperature change during calibration
Delay	delay set to each channel of the laser
Δ(D)	difference from previous delay setting
Power [mW]	power of the laser

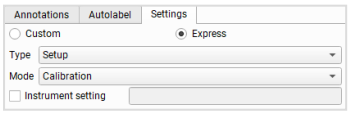
Table 1: Parameter of PMT calibration shown in analysis template

Manual PMT calibration

If the instrument is unable to read the barcode on the vial label of the MACSQuant Calibration Beads, a manual PMT calibration can be performed.

If the PMT calibration is started manually, i.e. without scanning calibration beads, the MACSQuant Instrument uses the reference median fluorescence intensities of the last scanned barcode to adjust the gains.

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 270 µL 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 calibration proceeds as described in the automated process. Refer to step 10 of the automated PMT calibration.

Troubleshooting PMT calibration

Calibration failed

A failed calibration may have the following causes.

1. High CV or low SI
   - Confirm that the optical bench warmed up for at least 30 minutes.
   - Ensure the correct running buffer in in the running buffer bottle holder.
   - Run several samples of 1% hypochlorite solution. Refer to the short instructions **Maintenance**. Repeat the PMT calibration.
   - Laser alignment may be needed. Contact Miltenyi Biotec Technical Support or initiate a MACSQuant Live Support session.
2. High noise
   - Release air from sheath particle filter when the instrument is in acquisition mode. Repeat the PMT calibration.
   - Check if the tubings from the running buffer bottle are tightly connected.
   - Check if the syringes behind the front door are tight. Refer to the chapter **Troubleshooting** in the **MACSQuant Instrument user manual**.
3. Out of range
   - Contact Miltenyi Biotec Technical Support or initiate a MACSQuant Live Support session.

Calibration incomplete

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

- Ensure to vortex the calibration beads thoroughly before use.
- Ensure the drop of the calibration beads reach the bottom of the tube.
- Repeat calibration after adding more calibration beads to the tube.
- Run a **Clean program**. Repeat the PMT calibration.
- Check robotic needle arm calibration. For more information, refer to the short instruction **Hardware calibration.**
- 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.

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