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.**

These short instructions are valid for the MACSQuantify Software 3.1 and later.

The reproducibility and stability of the fluorescence signal over time is of vital importance. To ensure a stable acquisition that is independent of time, the instrument must be calibrated. A daily calibration accounts for potential differences in laser performance or PMT sensitivity by adjusting the voltages accordingly. Calibrate the MACSQuant Instruments using MACSQuant Advanced Calibration Beads.
MACSQuant Advanced Calibration Beads contain blank beads, dim beads, bright beads, and three populations of single-stained fluorescent beads yielding intermediate signals in the target 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 on 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** dropdown list.
4 Vortex the MACSQuant Advanced Calibration Beads for 10 seconds to break up aggregates.
5 Click the **Barcode** button in the toolbar to activate the 2D barcode reader.
6 Place the bottle of compatible calibration beads in the right recess of the Single tube rack with the barcode facing the instrument.
7 Scan the 2D barcode printed on the vial label of the MACSQuant Advanced Calibration Beads. A window opens.
8 Select **Yes** to continue with the calibration.
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. See **Figure 1**.
To switch between the analysis windows, click the **Next** or **Previous** button.

Successful calibration is indicated for each fluorescence channel by a green check mark for the stain index **SI** and the coefficient of variation **CV**. The stain index of side and forward scatter is not taken into account as a factor for the calibration result. Furthermore, the table header of the calibration analysis page shows **Passed**, and the instrument status bar reports **Acquisition Mode: Calibration OK**. See **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 sign-in.

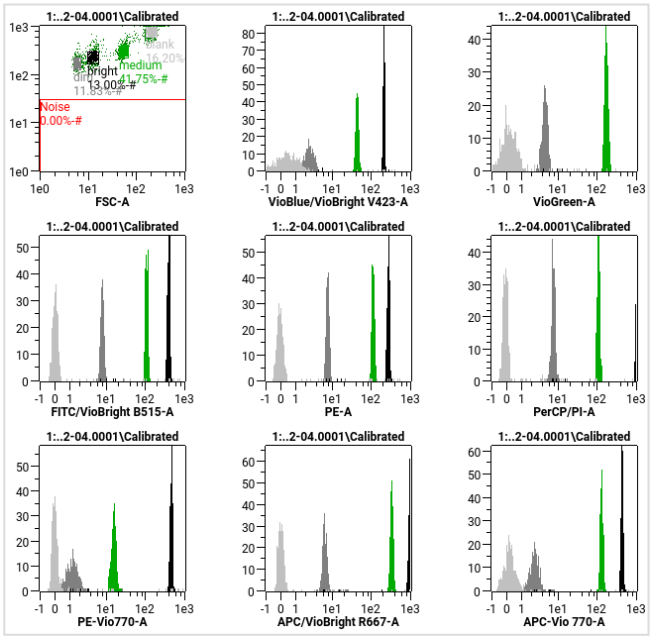



**Figure 1**: Calibration results of a MACSQuant Analyzer 10. Results look slightly different for other MACSQuant Instrument types.

| Parameter | Explanation |
|---|---|
| V | Voltage on PMT |
| Δ(V) | Difference from last successful calibration |
| SI | Stain index |
| CV | Coefficient of variation based on MFI |
| Δ(T) | Difference in temperature from the start of a calibration to the end of a calibration |
| Temperature range | Acceptable range of temperature change during calibration |
| Temperature | Measured temperature |
| Power range | Acceptable range of laser power |
| Power | Power of the laser |

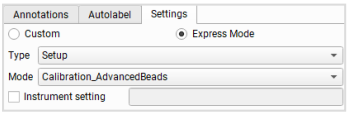
**Table 1**: Parameter of PMT calibration shown in analysis template

### Manual PMT calibration

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

If the PMT calibration is started manually, for example without the scan of calibration beads, one of the previously scanned calibration lot can be selected as reference for the manual calibration.

1. Ensure that the MACSQuant Instrument is primed and has been in acquisition mode for at least 30 minutes.
2. Click the **Experiment** tab.
3. Select **Single tube rack** from the **Rack** dropdown list.
4. Vortex the MACSQuant Advanced 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 Mode**.
9. In the **Type** dropdown list, select **Setup.**
10. In the **Mode** dropdown list, select **Calibration_AdvancedBeads**.




11. Select the calibration lot to be used as reference for the manual calibration.
12. Click the **Run** button in the instrument status bar.
    The calibration proceeds as described in the automated process. See 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 has warmed up for at least 30 minutes.
   - Ensure that the correct running buffer is 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.

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