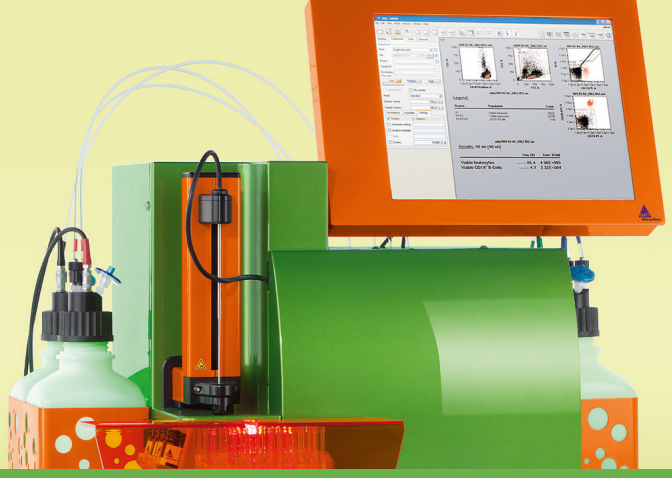


MACSQuant® Instrument short instructions

Photomultiplier tube calibration

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

Description

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 and instrument settings, the instrument needs to be calibrated. Fluorescence calibration curves are calculated based on the measurements of standardized fluorescent MACSQuant Calibration Beads with pre-defined size and fluorescence intensity.

As a quality control, the MACSQuant Instrument automatically adjusts voltage gains when performing photomultiplier tube (PMT) calibration with MACSQuant Calibration Beads to ensure that known fluorescence intensities are always set to the same channel.

Note: It is recommended to calibrate the instrument every other day.

Required materials

- MACSQuant Calibration Beads (# 130-093-607)
- 12×75 mm (5 mL) tube or microcentrifuge tube
- MACSQuant Running Buffer (# 130-092-747)

Automated PMT calibration

1 Ensure that the Single tube rack is correctly attached, and that the MACSQuant Instrument is primed and has been in acquisition mode for at least 30 minutes.

2 On the toolbar, click the **Barcode button** to activate the 2D code reader.

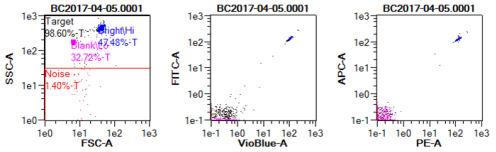
3 Scan the 2D barcode printed on the vial label of the MACSQuant Calibration Beads and follow the dialog box instructions.

4 Thoroughly vortex the MACSQuant Calibration Beads to break up any aggregates, dispense one drop into an empty tube, and place it in the Single tube rack.

5 Click **OK** to start the calibration. The calibration beads are automatically diluted to a total volume of 500 µL. 150 µL of the diluted calibration beads are injected into the sample injection port. During calibration, the gain for each respective channel is automatically adjusted.

6 The calibration results for each channel are presented as dot plots, histograms, and as a tabulated summary on a two-page (two-screen) report. Click the **Next window button** or **Previous window button** to switch between the screens.

7 Successful calibration for each channel is indicated by a green check mark. When the process is successfully completed, the MACSQuant Instrument Status bar reports **Acquisition Mode: Calibration OK**. All settings will be automatically saved as default settings.



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	486	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	15.84	0.15	1.08	✓	passed!

Manual PMT calibration

1 Ensure that the Single tube rack is correctly attached and that the MACSQuant Instrument is primed and has been in acquisition mode for at least 30 minutes.

2 Thoroughly vortex the MACSQuant Calibration Beads to break up any aggregates, dispense one drop into an empty tube, and place it in the Single tube rack.

3 In the side panel, go to the **Experiment tab**.

4 Go to the **Autolabel tab** to set the dilution and mixing of the calibration beads prior to calibration.

5 Click **<add…>** to open the Reagent dialog box.

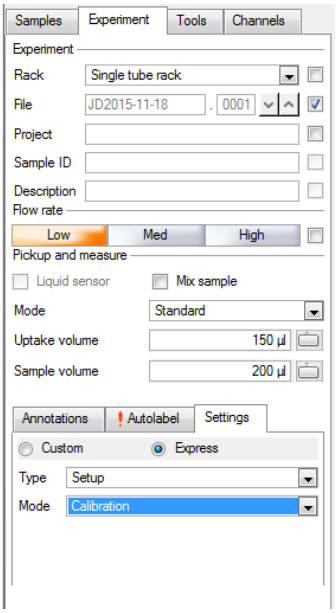
6 Select **S1** Special and Running Buffer A, B or C and adjust the dilution appropriately.

7 Set **Time** to 0 and **Titer** to 10:1, corresponding to a 10:1 dilution with no incubation time.

8 Close the **Reagents box** and check the box next to S1 Running Buffer A, B, or C.

9 Select the **Settings** tab and select **Express**.

10 Under **Type**, select Setup. Under **Mode**, choose **Calibration**.



11 Enter a sample volume of 50 µL.

12 Click the **Start Measurement button** in the instrument status bar. The calibration beads are automatically diluted to a total volume of 500 µL. 150 µL of the diluted calibration beads are injected into the sample injection port. During calibration, the gain for each channel is automatically adjusted.

13 Upon completion, an analysis template will indicate that the calibration has passed. Voltage gain, staining index, and fluorescence histogram plots are displayed.

14 The calibration results for each channel are presented as dot plots, histograms, and as a tabulated summary on a two-page (two-screen) report. Browse between the different screens by clicking the **Next window or Previous window buttons**).

Successful calibration for each channel is indicated by a green checkmark. When the process is successfully completed, the MACSQuant Instrument Status bar reports **Acquisition Mode: Calibration OK**. All settings will be automatically saved as the default settings.

Troubleshooting PMT calibration

Calibration failed

1. High CV for fluorescence channels
   - Confirm that the optical bench warmed up for at least 30 minutes.
   - Laser alignment may have drifted. Call Technical Service or initiate a MACSQuant Live Support session for assistance.
   - Run several samples of MACSBleach and then rerun calibration.
2. High noise
   - Check for air in pallfilter. Rerun calibration.
   - Run a **Clean program**. Rerun calibration.

Calibration incomplete

1. Not enough events acquired.

- Repeat calibration after adding more calibration beads to the tube.
- Check needle arm calibration. For more information, refer to the user manual.
- Go to **Edit > Options > Software > Acquire** and ensure that **Live events** is set to at least 5,000.
- Check that the trigger is not set too high. Move trigger down, save over default setting, and rerun calibration.
- Call Technical Service or initiate a MACSQuant Live Support session for assistance.

Are you in need of additional assistance?

Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

140-003-144.04