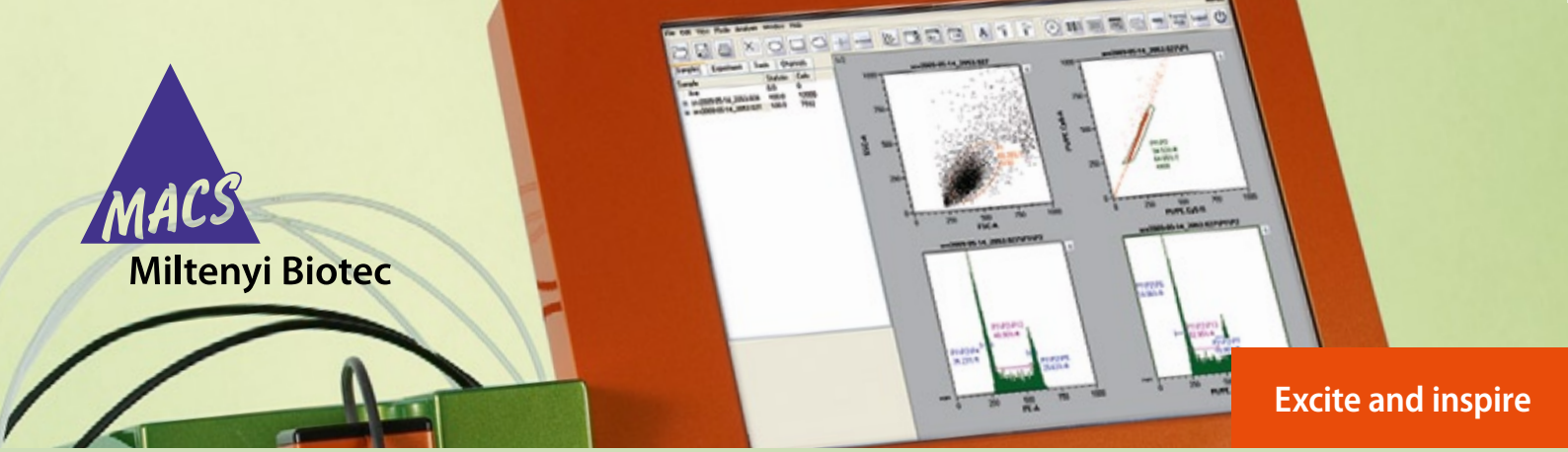

Miltenyi Biotec

Excite and inspire

Photomultiplier tube calibration

MACSQuant® Analyzer quick guide

Description

In flow cytometry, fluorescence intensity is used to distinguish between positive and negative populations of particles. The reproducibility and stability of the fluorescence signal is of vital importance, especially when performing comparable experiments over time. As a quality control, the MACSQuant® Analyzer automatically adjusts voltage gains when performing photomultiplier tube (PMT) calibration with MACSQuant Calibrarion Beads to ensure that known fluorescent intensities are always set to the same channel. This process is recommended to be performed 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)

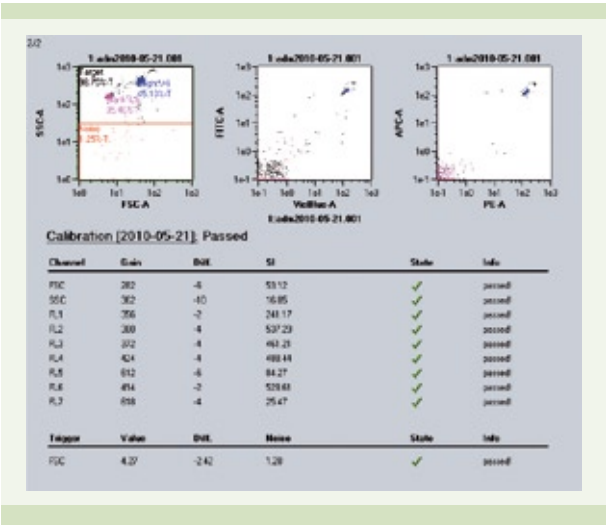

Figure 1: Calibration analysis template report

Automated PMT calibration

1. Prime the MACSQuant Analyzer and wait a minimum of 30 minutes for warm up of optics.
2. Thoroughly vortex the MACSQuant Calibration Beads to break up any aggregates.
3. Click the icon and present the vial barcode when the reader begins to flash.
   Note: The reader will take a few seconds to recognize the vial.
4. When the barcode is recognized, a dialog box will appear. Select '**Yes**' to proceed with calibration process.
5. Follow the on-screen instructions, i.e., place an empty tube into the attached single tube holder and place one drop of the MACSQuant Calibration Beads into it.
6. Click '**OK**' to start the calibration process.
7. The MACSQuant will automatically dilute the Calibration Beads and start measurement.
8. Upon completion, an analysis template will indicate that the calibration has passed. Voltage gain, staining index, and fluorescence histogram plots (figure 1) are displayed.
9. The MACSQuant Analyzer status bar should report 'Acquisition mode: Calibration OK'.

Manual PMT calibration

1. Prime the MACSQuant Analyzer and wait a minimum of 30 minutes for warm up of optics. Ensure single tube holder is correctly attached.
2. Thoroughly vortex the MACSQuant Calibration Beads to break up any aggregates.
3. In custom mode, select the '**Settings**' tab within the '**Experiment'** tab (figure 2).
4. In the '**Settings**' tab, click the '**Express**' radio button.
5. Select '**Setup**' from the '**Type**' drop-down menu and '**Calibration**' from the '**Mode**' drop-down menu.
6. Dilute one drop of the MACSQuant Calibration Beads in 500 µL of the MACSQuant Running Buffer in a 12×75 mm tube. Place the tube in the single tube holder.
7. Click the start measurement icon or push the orange button on the front of the single tube holder. This will start the calibration process.
8. Upon completion, an analysis template will indicate that the calibration has passed. Voltage gain, staining index, and fluorescence histogram plots (figure 1) are displayed.
9. The MACSQuant Analyzer status bar should report 'Acquisition mode: Calibration OK'.

(Optional) The MACSQuant Analyzer can perform pre-dilution and mixing of the MACSQuant Calibration Beads by setting preferences in the '**Autolabel**' tab. For details refer to section 3.6.2 of the user manual.

Troubleshooting PMT calibration

There could be multiple reasons why a calibration may not pass or be incomplete. Listed below are some common reasons.

Calibration failed.

1. High CV for fluorescence channels
   a. Confirm optical bench has warmed up for at least 30 minutes.
   b. Laser alignment may have drifted. Call technical service or initiate a MACSQuant Live Support session for assistance.
2. High noise
   a. Check for air in Pallfilter. Rerun calibration.
   b. Run 'Clean'. Rerun calibration.

Calibration incomplete

1. No events acquired.
   a. Check calibration of needle arm to washing station. For detailed information refer to section 3.5 of the user manual.
   b. Check that 'live events' in Edit > Options > Software > Acquire is set to at least 5,000.
   c. Check that the trigger is not set too high. Move trigger down, save over default setting, and rerun calibration.
   d. Call technical service or initiate a MACSQuant Live Support session for assistance.

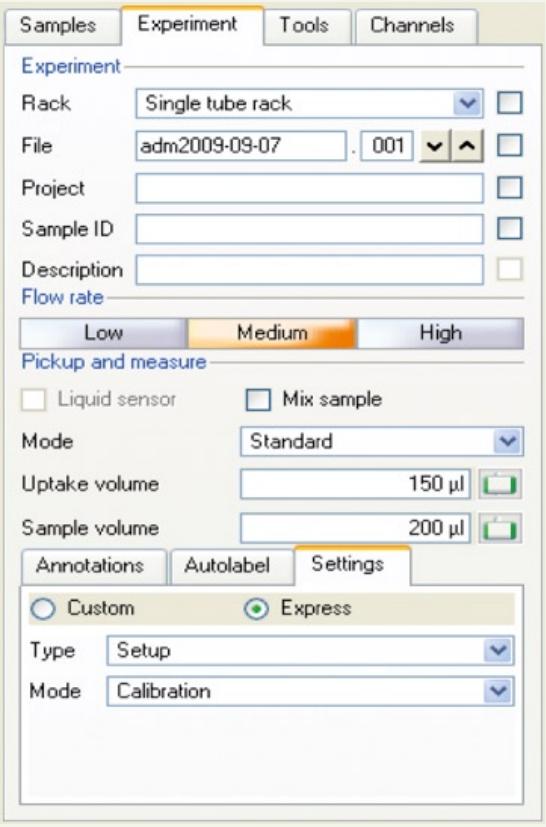

Figure 2: Experiment tab showing selection of calibration process in the settings tab

Are you in need of additional assistance?
Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

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