Miltenyi Biotec

MACSQuant® Instrument short instructions

Compensation

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

Introduction

These short instructions describe the setup of detectors for multicolor flow cytometry experiments. They also guide the user through the compensation process to correct fluorescence spillover.

Setup of detectors

1 Ensure that the MACSQuant Instrument is calibrated. Refer to the MACSQuant Instrument short instructions **PMT calibration**.
2 Open a new workspace via **File > New workspace**.
3 Open a new analysis window or a saved analysis template.
4 Acquire some unstained cells.
5 In the **Channels** tab in the sidepanel, adjust the voltage and scales for the light scatter channels so that cells of interest are visible in the FSC versus SSC plot.
6 For unknown samples or new stainings prepare and acquire single-stained controls. Ensure that

- populations are positioned on scale
- the resolution between negative and positive populations is good
- each fluorescent signal is brightest in its designated channel

7 Check all channels used in the experiment. Adjust the voltages if required.
8 Verify settings on a fully stained sample.
9 Select an appropriate trigger channel and value to remove debris or other unwanted populations of cells.
10 Save as an instrument setting.

Express Mode CompensationMultiColor

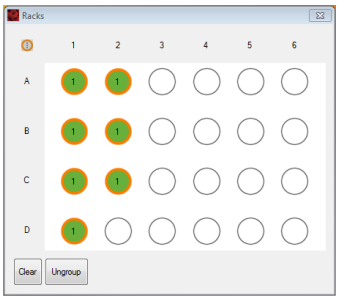
When performing a multicolor experiment, spillover from fluorescent channels into other channels can occur. The automated multicolor compensation corrects spillover for every channel.

Materials required

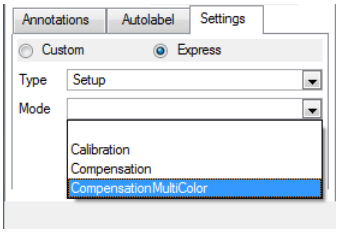
- Single-stained controls representing all fluorochromes used in the experiment. Use cells or compensation beads as controls.
- One unstained sample as blank for all samples or a negative population of unstained population in each tube.
- Optional: one unstained sample for compensation of viability dye channel, for example PI.
- 12×75 mm round bottom tubes or 96-well plate.
- MACS MiniSampler Plus with a Chill 5 or Chill 96 Rack or MACSQuant X Orbital Shaker with a MACSQuant X 5 Rack or 96-well plate.

Protocol

1 Go to **File > New workspace** to open an empty workspace.
2 Load the instrument setting from setup of detectors.
3 Select an appropriate sample rack from the drop-down menu in the **Experiment** tab.
4 Click the **Rack** button in the toolbar.
5 Select the appropriate number of sample positions to match the number of samples. Note the rack processing order (see symbol in the upper left corner).
6 Place your samples in the rack accordingly.



7 Click the **Group** button.
8 Go to the **Experiment** tab.Select the **Settings** tab and activate the **Express** radio button.
9 Select **Setup** from the **Type** drop-down menu.
10 Select **CompensationMultiColor** from the **Mode** drop-down menu.



11 Select a single sample position in the rack window. Select the respective fluorochrome from the **Sample ID** drop-down menu matching the order of the samples in the rack.

12 Each fluorochrome in the drop-down menu represents a specific detection channel (e.g. FITC represents channel B1). If using another B1-compatible fluorochrome, e.g. Vio B515, select FITC.
13 Optional: If one blank sample is used for all stained controls, then select **Blank** from the drop-down menu.
14 Select **PI** from the drop-down menu for compensation of viability dyes detected in channel B3. Do not add any staining. Only spectral overlap into the viability channel will be compensated.
15 **Current Software version 2.13.2 or later** : Select **7AAD**, **PI**, or **other viability dye** from the drop-down menu for compensation of viability dyes. If **other viability dye** is selected, enter the fluorescence channel, e.g. R1, into the **Description** field. Do not add other information. Do not add any staining into the tube.
16 Optional: Highlight all positions of the sample rack. Select mixing, change the flow rate, change the mode, or change the uptake and sample volume if required.
17 Check the levels of MACSQuant Running Buffer and the waste container.
18 Click the **Run** button to start the acquisition. Follow the instructions on the screen.
19 Select the **Settings** icon in the toolbar. Select the **Compensation** tab in the **Instrument settings** window to review the compensation matrix when compensation is completed.

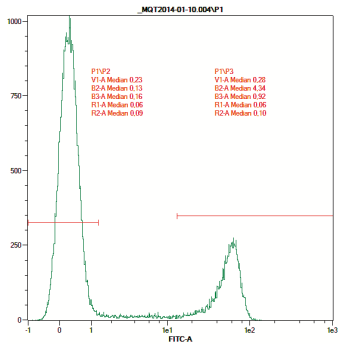
Manual compensation

Materials required

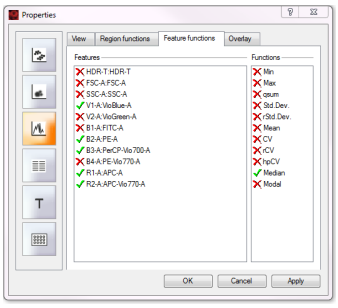
- Single-stained controls representing all fluorochromes used in the experimental staining panel. Each sample must contain a negative and a positive population.
- 12×75 mm round bottom or 1.5 mL tubes.
- Single tube rack.

Protocol

1 Ensure that the MACSQuant Instrument is calibrated.
2 Load the instrument setting from setup of detectors.
3 Select an analysis template by clicking on the **New analysis window** button in the toolbar.
4 Change one dot plot into a histogram, and display the respective channel of the stained sample (e.g., use B1 for FITC).
5 In the **Experiment** tab, define parameters, e.g. mixing, **Sample ID** and **Uptake volume**.
6 Open the compensation matrix by clicking the **Instrument settings** icon in the toolbar. Select the **Matrix** checkbox.
7 Place the single-stained sample into the Single tube rack.
8 Click the **Run** button.
9 When events start to appear on the plots, pause the measurement: Right-click on the **Stop** button and click the **Pause** button.
10 Draw a region around the population of interest within the FSC versus SSC dot plot. This will be P1.
11 Select P1 from the plot header of the histogram plot.
12 Draw a range region around the negative (P2) and positive population (P3).



13 Click the **i** button.
14 Go to the **Region Functions** tab and select P2 and P3.
15 Go to the **Feature Functions** tab to simultaneously show the medians for all channels used in the experiment. Select the respective channels and select **Median**.
16 Click **OK**.



17 Click the **Pause** button to resume measurement.
18 To adjust compensation, select the appropriate cell in the matrix. The columns represent the measured fluorochrome, the rows represent the detection channels. For example, to correct the spillover of a FITC-stained sample into the PE channel, go to the cell FITC/B2.
19 Adjust the values until the median fluorescence for the positive and negative populations are equal.
20 Once the compensation is adjusted for one fluorochrome, continue with compensation for all other fluorochromes.
21 When finished, save as an **Instrument setting** file.

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.

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