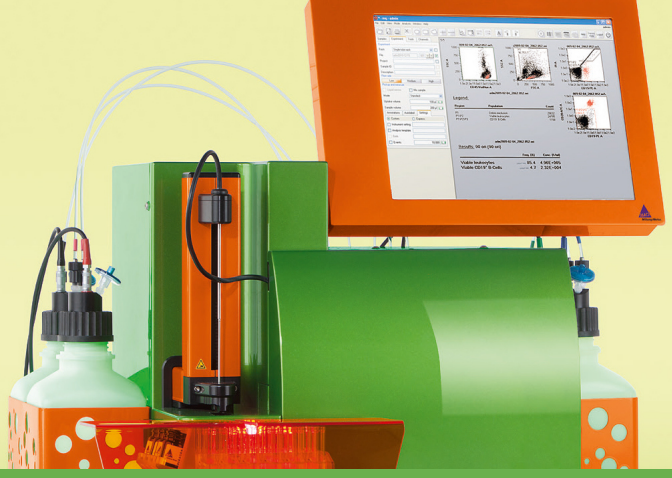
Miltenyi Biotec



MACSQuant® Instrument short instructions

Instrument settings

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

Setup of detectors

1 If necessary, perform a PMT calibration (refer to the MACSQuant Instrument short instructions **PMT calibration** for details).

2 In a new workspace, open a new analysis window, or a saved analysis template and acquire some unstained cells.

3 In the Channels tab in the sidebar, adjust the voltage and scales for the light scatter channels so that cells of interest are visible in the FSCvSSC plot. If fluorescent channels require a different voltage setting than default, use a fluorescently stained cell sample to ensure populations are positioned on scale.

4 To remove debris, or other populations of cells not needed within the dataset, select an appropriate Trigger channel and value.

Note: Details on manual detector adjustment can be reviewed in the MACSQuantify™ Software guide.

Automated multicolor compensation

Materials required

- Single-stained controls representing all fluorochromes to be used in the experimental staining panel. Ensure that there is a comparable positive and negative population for setting compensation.
- One unstained sample for use as blank sample and another unstained sample for compensation against PI staining (optional).
- 12×75 mm round bottom tubes or 96-well plate
- MACS MiniSampler Plus with either Chill 5 or Chill 96 Rack (MACSQuant Analyzer 10, VYB, Analyzer 16) or MACSQuant X Orbital Shaker with 96 well plate (MACSQuant X).

Protocol



1 Ensure that the Instrument is calibrated and that all PMT voltages for the cell sample are adjusted.

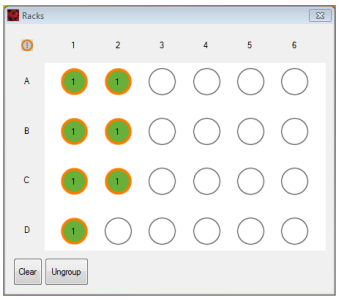
2 Prepare single-stained controls for all fluorescent reagents used in the experimental staining panel. Cells or compensation beads can be used as controls.

Note: It is recommended to have one tube that is a true blank for the compensation controls.

3 Select an appropriate Chill rack from the drop-down list in the **Experiment tab**. Typically, the Chill 5 or the Chill 96 rack is used.

4 Click on the **Rack button** (1) in the toolbar, if the **Rack dialog box** does not automatically appear.

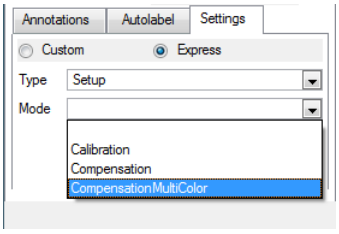
5 In the **Racks dialog box**, the respective rack will be displayed. Select the appropriate number of sample positions to match the number of samples that will be used for compensation. Note the rack processing order (horizontal or vertical, see symbol in the upper left corner) and place your samples in the rack accordingly.



6 Click on the **Group button** at the bottom of the window. Each of the selected rack positions should now be labeled with the number 1.

7 Go to the **Experiment tab**. Click on the **Settings tab** and activate the **Express option button**.

8 From the **Type drop-down list**, select **Setup**. From the **Mode drop-down list**, select **CompensationMultiColor**.



9 Select an individual sample position in the rack window (only one at a time). In the **Sample ID field**, a drop-down list will become available. Select the respective fluorochrome.

10 Each fluorochrome that appears in the drop-down list represents a specific detection channel (e.g. PerCP-Vio770 represents channel B3). If using another B3–compatible fluorochrome, e.g., PE-Cy5, PerCP-Vio770 must be selected.

11 Select **Blank** for a sample of unstained cells. This is the reference for negative cellsand should always be included.

12 Select PI if propidium iodide is used for dead cell exclusion in later experiments. It is used for correcting all fluoresecence spillover from the B3 channel. Addition of PI to this tube is not necessary.

> **Note:** For compensation, use an unstained sample that matches other compensation controls and do not add PI.

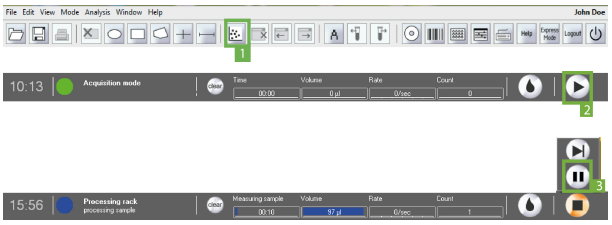
13 Place samples in proper rack positions and start the acquisition. Follow the onscreen instructions.

Manual compensation using the 8×8 compensation matrix

Materials required

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

Protocol



1 Ensure the MACSQuant Instrument is calibrated, and that all PMT voltages for the cell sample are adjusted.

2 Choose an analysis template (e.g., four dot plots) by clicking on the **New analysis window button** (1) in the toolbar.

3 Change one dot plot into a histogram and display the respective channel of the stained particles (e.g., use B1for FITC).

4 In the **Experiment** tab, set all necessary parameters, e.g. **Sample ID** and **Uptake volume**.

5 Open the compensation matrix by clicking the Instrument settings button in the toolbar or under **Edit > Instrument settings** and selecting the **Compensation** tab.

6 Click the **Start Measurement button** (2).

7 When events start to appear on the plots, pause the measurement: Right-click on the **Stop button** in the instrument status bar and click the **Pause button** (3).

8 Draw a scatter region around the population of interest within the FSC versus SSC dot plot. This will be P1.

9 Display events in P1 region in the fluorescence histogram plot by selecting the P1 region from the drop-down menu of the plot header.

10 Draw interval regions on the negative and positive populations.

11 Display the medians for all spillover channels used in the experiment by clicking on the **i** button, selecting the spillover channels (e.g. B2, B3, etc) , and **Median** from the **Feature Function tab**.

12 Click on the **Pause button** (3) to resume measurement.

13 Click the **Matrix** checkbox.

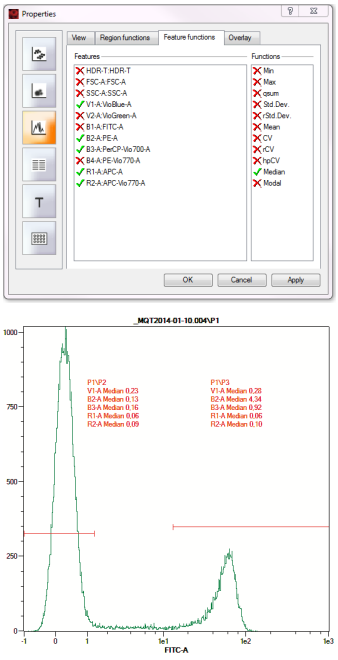
14 Place the first single-stained tube (e.g., FITC-stained cells) into the single tube holder.

15 To add compensation to the combination of channels in the plot, choose the appropriate cell in the matrix. The columns represent the measured fluorochrome, the rows represent the detection channels, where the spillover fluorescence should be corrected. E.g., to compensate a FITC-stained sample against the PE channel, go to the cell FITC/B2 and adjust the value to achieve equivalent median fluorescence intensities values.

16 Adjust values for other spillover channels, if necessary.

17 Once compensation is adjusted for this fluorochrome, repeat compensation for all additional fluorochromes.

18 When finished, save as an **Instrument settings** file.



Miltenyi Biotec

Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. MACS, MACSQuant, MACSQuantify and the Miltenyi Biotec Logo are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.
Copyright © 2020 Miltenyi Biotec and/or its affiliates. All rights reserved.

140-003-146.05