

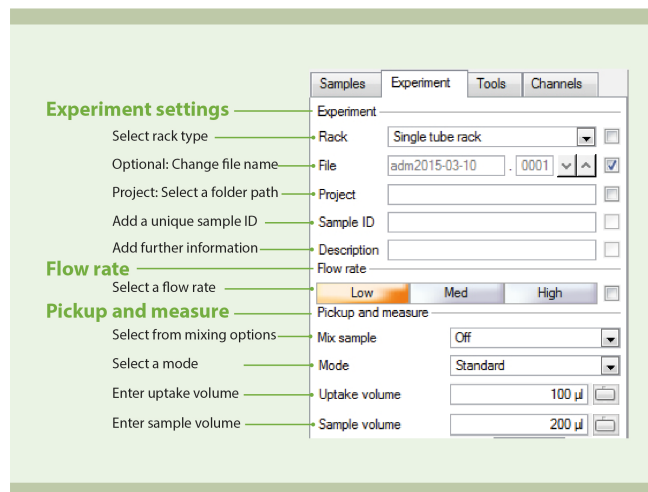
MACSQuant® Instrument short instructions

Setting up an acquisition

Setting up an experiment on the MACSQuant® Instrument

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

The MACSQuantify™ Software provides the user a simple interface to program various sample parameters for data acquisition. Follow this quick guide to see how to easily set up a basic experiment for acquisition in the single tube format.



The screenshot shows the 'Experiment' tab in the MACSQuantify software. The interface includes sections for 'Experiment settings', 'Flow rate', and 'Pickup and measure'. Key settings visible include: Rack (Single tube rack), File (adm2015-03-10 - 0001), Project, Sample ID, Description, Flow rate (Low, Med, High), Mix sample (Off), Mode (Standard), Uptake volume (100 µL), and Sample volume (200 µL).

Figure 1: The Experiment tab.

General experiment settings

- 1 Go to the **Experiment** tab.
- 2 From the **Rack** drop-down list, select **Single tube rack**. If the box to the right of the field is activated, the instrument will automatically detect the used rack. Available rack types differ between instrument types. .
- 3 (Optional) Change the file name: deactivate the **File** checkbox to the right of the file name and enter a unique file name.
- 4 Enter alphanumeric text for the **Sample ID** and **Description** if desired.

Flow rate

Keep events/second rate below 10,000 for accurate measurements. 1000 - 5000 events/second is ideal. The flow rate can be adjusted by doing one of the following:

- Select **Low** for high cell densities ($\sim 10^7$), **Medium**, or **High** for very low cell densities ($\sim 10^6$).
- Check the box to the right to switch to events/s. The flow rate is automatically adjusted to approximate the selected value.

Flow rate (µL/min)	Sheath buffer consumption (µL / measurement)	Total time (s)
Low: 25	18,000	240
Med: 50	13,000	120
High: 100	8,600	60

Table 1: Flow rate and sheath buffer consumption for a 100 µL sample, run in standard mode.

Pickup and measure

- 1 From the drop-down menu **Mix sample**, select a mixing option if it is desired to premix the sample before sample pickup, data acquisition and analysis. Available mixing options differ between the instrument types.
- 2 Select a mode from the **Mode** drop-down list (Screen, Fast, Standard, Extended, Enrich, EnrichS, EnrichS2).

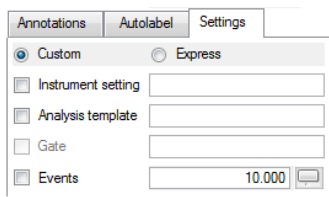
Note: Enrich programs are only available on MACSQuant 10 or VYB instruments. Screen is only applicable if using a chill rack.

- 3 Enter an **Uptake volume** and a **Sample volume**. The maximum uptake volume and sample volume for the MACSQuant 10/VYB is 450 µL and 5 mL, respectively. The maximum uptake volume and sample volume for the MACSQuant X is 5 mL.

Note: If mixing is selected, it is necessary to accurately define the sample volume to avoid the uptake of air bubbles.

Settings

- 1 Go to the **Experiment** tab and select the **Settings** tab.
- 2 Activate the radio button next to **Custom**.



- 3 For Custom mode analysis, select the checkboxes next to **Instrument settings**, **Analysis template**, **Live gate**, and **Events options** to activate these features. When deselected, the instrument will run with the current active Instrument settings.
 - **Instrument setting:** select the adjacent checkbox to load and apply previously saved instrument calibration and compensation settings.
 - **Analysis template:** select the adjacent checkbox to load and apply previously saved cell analysis templates.
 - **Gate:** select the adjacent checkbox to activate gate settings. **Stop gate:** measurement will end and datafile will be saved once event limit is reached in the chosen gate. **Live gate:** only events within the gate will be saved in the datafile.
 - **Events:** select the adjacent box to stop data acquisition and save datafile after a defined number of total events is obtained; in this example, 10,000 events.

Plot properties

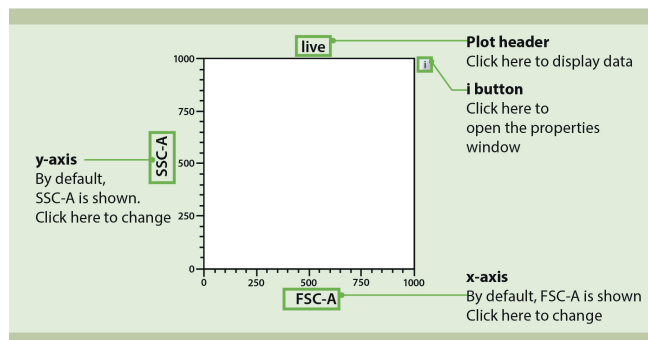


Figure 2: Features of a MACSQuantify™ plot. To modify its appearance, click on the 'i' button to the right of the plot.

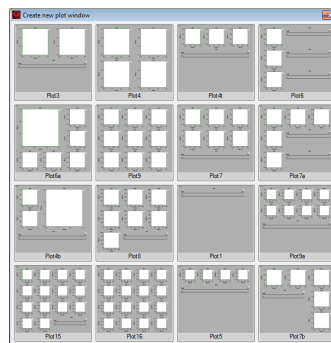
Open an analysis window

Analysis templates consist of a plot template and, if required, a gating strategy. Analysis templates can be created post-acquisition or during acquisition in Live mode. The next steps are NOT necessary if a pre-defined analysis template is already selected within the **Settings** tab, or opened within the workspace.



- 1 Click the **New analysis window button** (1) in the toolbar or go to **Window > New analysis window**.

- 2 Select the desired analysis template from the plot options. Multiple analysis windows can be opened, each can be comprised of single or multiple experiments.



- 3 If multiple analysis windows are open, use the **previous and next analysis window buttons** (2) in the toolbar to access hidden windows.

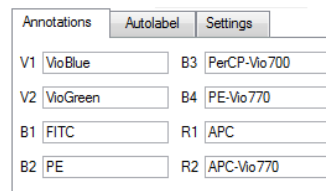
Draw a gate



- 1 To create a gate or region, click on one of the **tool buttons** (1) from the toolbar or choose the tool from the **Edit** menu.
- 2 If using ellipse, rectangle, quadrant or interval, left-click on a plot and start dragging a gate. Release the left button to finish drawing.
- 3 If drawing a polygon, left-click once to start drawing the first point. Move the cursor and left-click again to draw the next point. Continue as desired. Double-click to draw the last point and finish the gate.
- 4 When the gate is selected, the edit points can then be used to adjust the size and shape.
- 5 Click and drag the gate to move it to a new position.

Annotation

- 1 Select the **Annotations** tab.
- 2 Modify the annotations for the fluorescence channels, if required.



Note: Annotations may not appear on the plot display window until acquisition starts.

Are you in need of additional assistance?

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