



MACSQuant® Instrument short instructions

Setting up an acquisition

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

Introduction

The MACSQuantify Software provides the user a simple interface to program various sample parameters for data acquisition. Review this short instruction to set up a basic experiment for acquisition in the single tube format.

Experiment tab

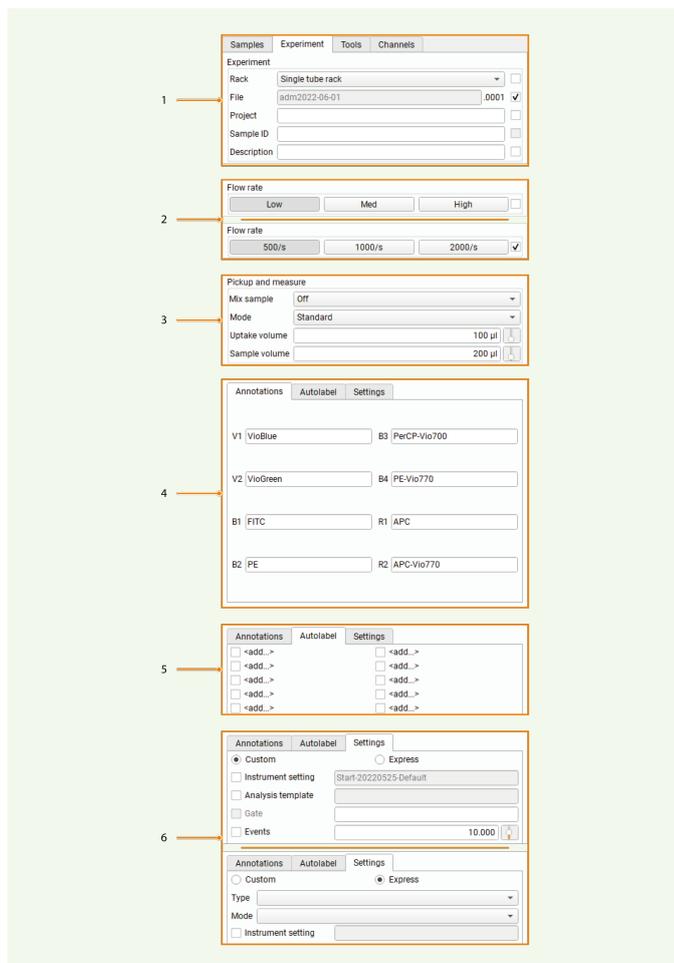


Figure 1: The **Experiment** tab options of a MACSQuant Analyzer 10

Use the sections of the **Experiment** tab to program your sample acquisition.

Experiment (1)

- 1 Go to the **Rack** drop-down menu and select **Single tube rack**.
- 2 Optional: Change the name of the file. Clear the box to manually change the file naming. Use alphanumeric text only. A selected checkbox indicates automated file naming.
- 3 Optional: Enter a name for a project folder in the field **Project**. All data are saved in this folder. Select the box next to **Project** to see a drop-down menu of all project folders of the logged in user.
- 4 Optional: Enter alphanumeric text as sample ID and description.
- 5 Select the box on the right-hand side to **Description** to determine an automated pattern. Refer to the **MACSQuantify Software user manual**.

Flow rate (2)

Keep the event rate below 15,000 events per second for accurate measurements. 1000–5000 events/s is ideal.

The flow rate can be adjusted by doing one of the following:

- Select **Low**, **Medium**, or **High** depending on the cell concentration and research question. Select **Low** for high cell concentrations, for example, 10^7 cells/mL.
- Select the box on the right-hand side to switch to events per second. The flow rate is then automatically adjusted to low, medium, or high during acquisition to approximately reach the selected event rate.

Pickup and measure (3)

- 1 Go to the drop-down menu **Mix sample** to select a mixing option. Available mixing options differ between the instrument types.
- 2 Select a mode from the **Mode** drop-down menu. Available modes differ between the instrument types. The selected mode defines the washing steps between samples or during enrichment using the MACSQuant Column.
- 3 Enter an uptake volume and a sample volume. The maximum uptake volume is 450 μ L on the MACSQuant Analyzer 10, VYB, and Analyzer 16, and 5 mL on the MACSQuant X.

If mixing is selected on a MACSQuant Analyzer 10, VYB, or Analyzer 16, it is necessary to accurately define the sample volume to avoid the uptake of air bubbles.

Annotations tab (4)

- 1 Click into the field of a channel to change its annotation.

Annotations	Autolabel	Settings
V1 VioBlue	B3 PE-Vio615	
V2 VioGreen	B4 PerCP	
V3 BV570	B5 PerCP-Vio700	
V4 BV605	B6 PE-Vio770	
V5 BV650	R1 APC	
B1 FITC	R2 Alexa Fluor700	
B2 PE	R3 APC-Vio770	

If using a saved instrument setting the annotations are reset to the annotations of the saved instrument setting.

Autolabel tab (5)

Automated sample labeling can be activated in the **Autolabel** tab. For single tube experiments, only automated dilution of samples is possible. Refer to the short instructions **Multisampling and autolabeling** for autolabeling of multiple samples.

Settings tab (6)

Use the **Settings** tab to select the **Custom** radio button to set up a customized acquisition, or select the **Express** radio button to run an Express Mode.

Custom

- 1 Select the **Custom** radio button.

Annotations	Autolabel	Settings
<input checked="" type="radio"/> Custom	<input type="radio"/> Express	
<input type="checkbox"/> Instrument setting		
<input type="checkbox"/> Analysis template		
<input type="checkbox"/> Gate		
<input type="checkbox"/> Events	10.000	

- 2 Select the checkboxes next to **Instrument setting**, **Analysis template**, **Gate**, and **Events** to activate these features. When cleared, the instrument runs all samples with the current active instrument setting and analysis template.

The annotations in the instrument setting file override any annotations set in the experiment tab.

Annotations	Autolabel	Settings
<input checked="" type="radio"/> Custom	<input type="radio"/> Express	
<input checked="" type="checkbox"/> Instrument setting		
<input type="checkbox"/> Analysis template		
<input checked="" type="checkbox"/> Gate	live: <input type="checkbox"/> P1	
<input checked="" type="checkbox"/> Events	10.000	

- **Instrument setting:** Select the checkbox to use saved instrument settings.
- **Analysis template:** Select the checkbox to use saved analysis templates.

- **Gate:** Select the checkbox to activate gate settings. A gate must be drawn in a plot to enable selection of the checkbox. **Stop gate:** Used in combination with a defined event limit. When the event limit is reached in the selected gate, the measurement stops. Data are saved automatically including events outside the stop gate. **Live gate:** Only events within the selected gate are saved in the data file. Can optionally be used in combination with a defined event limit.
- **Events:** Select the checkbox to stop data acquisition and save the data file after a defined number of total events were acquired. Can also be used in combination with a **Stop gate** or **Live gate**. Values in events are represented by European numbering.

Express

- 1 Select the **Express** radio button.
- 2 Select **Setup** from the **Type** drop-down menu to use an Express Mode for calibration or compensation processes.

Annotations	Autolabel	Settings
<input type="radio"/> Custom	<input checked="" type="radio"/> Express	
Type: Setup		
Mode:		
	Calibration	
	TubeLengthCalibration	
	VolumeCalibration	
	Compensation	
	CompensationMultiColor	

- 3 Select **Analysis** from the **Mode** drop-down menu to use Express Modes for analysis of cells with Miltenyi reagents such as MACS Antibody Cocktails or MACSPlex Kits. Experiment settings such as mixing, mode, uptake volume, and sample volume are loaded automatically.
- 4 Optional: Change the experiment settings.
- 5 Optional: Enter a **Sample ID** and **Description**.
- 6 For grouped Express Modes, select the sample ID from the **Sample ID** drop-down menu.
- 7 Start the measurement. The acquisition page of the Express Mode is shown automatically.
- 8 The Express Mode analysis is performed and automatically shown after measurement of the current sample.



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