

MACSQuant[®] Instrument short instructions

Multisampling and autolabeling

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

Introduction

Use the MACSQuant Analyzer 10, VYB, or Analyzer 16 in combination with the MACS MiniSampler Plus and one of the Chill Racks to measure multiple samples. Use the MACSQuant X in combination with the MACSQuant X Orbital Shaker and the MACSQuant X 5 Rack or appropriate plates to measure multiple samples.

Different sample racks are available, allowing for processing up to 96 (MACSQuant Analyzer 10, VYB, or Analyzer 16) or 384 samples (MACSQuant X) in a single experiment. Different types of cell samples or analysis panels can be configured on the same rack. Samples can be automatically labeled with fluorochrome-conjugated antibodies, cocktails, or fluorescent dyes prior to measurement. Use the Universal Reagent Rack for labeling samples with up to four reagents. Miltenyi Biotec reagents can be entered automatically using the 2D barcode reader.

Selecting a rack

- 1 Go to the **Experiment** tab.
- 2 Select the required rack type from the **Rack** drop-down menu.

Only calibrated racks and related ones are shown in the drop-down menu.

A scheme of the selected rack is shown at the bottom of the **Experiment** tab.

Programming sample positions

Program sample positions in the **Experiment** tab. Selected samples indicated by an orange rim can be edited. Selected samples can be programmed at the same time, or each position or group of positions can be programmed individually.

- 1 Select the sample position(s).
- **2** Optional: right-click a number or letter to select an entire column or row.

Rack	3					×
$\downarrow \downarrow$	1	Select col		5	6	
Α		Select used in col)			
в		Deselect col				
		Clear col	Ľ			
С		Clear selected in col	$\left(\right)$			
		Clear unselected in col				
0						
Clear	Group					

- 3 Adjust the Experiment, Flow rate, and Pickup and measure options.
- 4 Go to View > Experiment table to check the experiment settings for all sample positions.

Refer to the MACSQuant Instrument short instruction **Setting up an acquisition** or to the **MACSQuantify Software user manual** for more information.

Refer to **Table 1** for an overview of the sample position color code and to the **MACSQuantify Software user manual** for more information.

Sample position color code

Clear	\bigcirc	Default open circle indicates no operation.
Closed green circle		Sample used for measurement. Click circle to select for editing.
Closed green circle with orange rim		The orange rim indicates that the sample is selected and activated for editing.
Closed yellow circle	•	Processing of sample has started, for example, sample is diluted and incubated.
Closed blue circle with orange rim		Measurement is in progress.
Closed gray circle		Measurement has been finished.

Table 1: Sample position color code

Autolabeling

- 1 Go to the **Experiment** tab.
- 2 Select a multisample rack type.
- 3 Go to the Autolabel tab.
- 4 Select any **<add...>** box to open the **Reagents** window.

Annotations	Autolabel	Settings		
<add></add>			add>	
<add></add>			add>	
<add></add>			add>	
<add.< td=""><td></td><td>· · ·</td><th>add></th><td></td></add.<>		· · ·	add>	
<add></add>			add>	

- **5** Select the reagent and Universal Reagent Rack position from the dropdown menu of the **Reagents** window.
- **6** Optional: Scan the Miltenyi Biotec Reagents with the 2D barcode reader to automatically load them into the reagents list.

Pos	Category		Reagent		Time	Titer	Order	
✓ R1	Universal	٣	Propidium Iodide Solution	*	0 .	1:100 💌 🔺	8 .	i
✔ R2	Human Cocktails	*	MC CD14 Monocyte Cocktail, human	*	20	1:11 • •	5 * *	i
🗌 R3	Calibration	Ŧ	MACSQuant Calibration Beads	w		1:100 -	5 .	i
🗌 R4	Calibration	Ŧ	MACSQuant Calibration Beads	Ŧ	0	1:100 -	5 -	i
√ S1	Special	¥	Running Buffer A	٠		10:1 • •	5 .	i
S2	Special		Running Buffer A	Ŧ	0	10:1 × *	5 -	i

Annotations are automatically set when using selected Miltenyi reagents in the **Settings** tab.

- Optional: To dilute samples automatically by the MACSQuant Instrument prior to measurement, select a buffer dilution from S1 or S2. A total of four reagents plus dilution with buffer can be added.
- 8 Adjust the incubation time, titer, and order as required. MACS MC Cocktails and fluorochrome conjugated antibodies have predefined times and titers.

The order is important if several reagents are added to one sample. Reagents are added starting with the lowest number. The titer defines the dilution of the sample based on the sample volume.

Changed titers or times appear in red font.

- 9 Click Apply to confirm.
- 10 Go to the Rack window to select one or more wells for autolabeling.
- 11 Go to the Experiment tab and select the Autolabel tab.
- 12 Select the reagent(s) to be added to the selected wells.

A red exclamation mark indicates that autolabeling is activated.

Annotations	Autolabel	Settings	
V PI		<add< th=""><th>L.></th></add<>	L.>
MC CD14, h		<add< th=""><th>l></th></add<>	l>
<add></add>		<add< th=""><th>L.></th></add<>	L.>
<add></add>		<add< th=""><th>L.></th></add<>	L.>
Running Buffer	Α	<add< th=""><th>></th></add<>	>

13 Repeat for other samples and reagents if required.

It is possible to assign a predefined analysis template to a sample position when using analysis cocktails such as CD14 Monocyte Analysis Cocktail, anti-human, for autolabeling. Select the corresponding Analysis Express Mode in the **Settings** tab, for example, MC_CD14_h.

Reviewing experiment settings

All experiment settings can be reviewed in the experiment table.

- 1 Go to View > Experiment table.
- 2 Switch between Acquisition, Annotations, Autolabel, or Settings to review the respective settings.

cquisition	Annotation	ns Aut	olabel Settings	s				
Sample I	D Description	Flow rate	Auto flow rate on	Mix sample	Mode	Uptake volume	Sample volume	Description Fragment
A1	Comp	Med	No	Mix medium	Standard	50	200	Comp
B1	Comp	Med	No	Mix medium	Standard	50	200	Comp
C1	Full stain	Low	No	Off	Standard	100	200	Full stain
D1	Full stain	Low	No	Off	Standard	100	200	Full stain
A2	Full stain	Low	No	Off	Standard	100	200	Full stain

Before starting the measurement, check the following:

- The instrument is correctly calibrated.
- The correct instrument setting, including compensation, is selected.
- Experiment definitions are correctly assigned to each sample position and each sample is correctly positioned on the Chill Rack.
- Sufficient quantities of reagents and buffers are provided. Ensure that the waste bottle is empty.
- Reagents are positioned correctly on the Universal Reagent Rack.
- Caps of the reagents are removed.



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