Miltenyi Biotec



MACSQuant® Tyto® instrument short instructions

Setting up a sort workspace

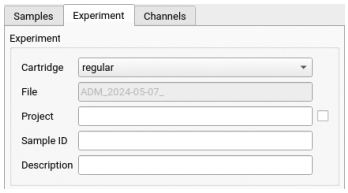
These short instructions are valid for MACSQuantify™ Tyto Software 3.2 and later.

The MACSQuant Tyto Instrument workspace manages all aspects of the cell sort. It includes the analysis template, experiment parameters for a particular sort, instrument settings, and allows monitoring of the sort.

Defining experiment parameters

General experiment settings

1 Go to the **Experiment** tab.



2 To set the cartridge type, scan the 2D barcode of the cartridge by presenting it to the 2D barcode reader window. Alternatively, select the cartridge type using the **Cartridge** dropdown list.

3 Enter alphanumeric text for the **Project**, **Sample ID**, and **Description** if desired.

4 MACSQuant Tyto Lux Cell Sorter only: Select the **Laser mode**.

5 Enter the volume to be processed by the instrument in the **Processing volume** box. Press enter on the keyboard.

6 After creating an analysis template (see **Creating an analysis template**), select the desired gate as sort gate from the **Sort gate** dropdown list or right-click on the desired gate in a plot and click **Make sort gate**.

7 To set the sort mode, use the slider.
It is recommended to enable coincidence aborts when using the sort mode **High Purity** or **Ultra Purity**.

8 To enable coincidence aborts, select the checkbox.
Statistics for coincidence aborts are shown in the table of the **Real time sort statistics** window.

9 Modify the annotations for the fluorescence channels, if required.

Mixer

The mixer of the cartridge keeps the cells in the sample in suspension. If required, adjust the propeller parameters of the cartridge in the mixer section of the **Misc Controls** tab on the touchscreen. The preset mixer speed of 800 rpm is recommended for most cell types.

Automated priming

The automated priming feature enables automated cartridge priming on the instrument. Select the checkbox **Automated Priming** in the **Misc controls** tab on the touchscreen to activate automated priming, or prime manually. Refer to the **MACSQuant Tyto instrument short instructions Cartridge priming and sample loading**.

Chamber temperature

The temperature of the cooling chamber can be set. Enter a target temperature for the cooling chamber (4 to 25 °C) in the temperature section of the **Misc Controls** tab on the touchscreen.

Creating an analysis template

1 Click the **New analysis window** button on the toolbar or go to **Window > New analysis window**.

2 Select the desired analysis template from the plot window options. Multiple pages can be chosen to match the plot layout necessary for the analysis template.

3 Click the **'i'** button in the upper right corner of the plot to change its plot type and properties. Click the axis label to modify the shown parameters.

4 Select a gating tool from the toolbar.

5 Create a hierarchical gating strategy, if necessary (**Figure 1**).

6 Draw a gate (P1) in the plot.

7 Click the plot header of the next plot and select P1.
Only events of P1 are shown.

8 Draw a gate (P2) within the plot showing the events from P1.

9 To select a sort gate, right-click on the desired gate in a plot and click **Make sort gate**.

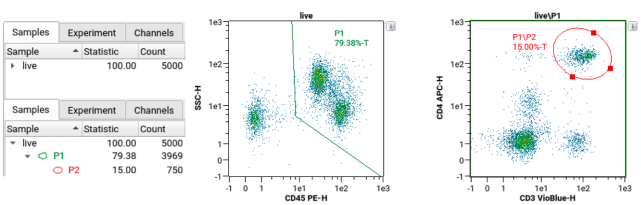


Figure 1: Example of a hierarchical gating. Click the **Arrow** icon next to the sample in the **Samples** tab to open the gating hierarchy. The hierarchy level, gate type, name, and color are shown in the hierarchy tree.

Adjusting the event detection settings

The event detection of the MACSQuant Tyto Instrument is based on the backscatter signals. To adjust the PMT voltages and thresholds, it is recommended to use the sort sample. It should contain a negative and a positive population for each of the used flourochromes of the sort panel. Avoid increasing PMT voltages above 700 V.

When using the **High Purity** or **Ultra Purity** mode keep the trigger values at the minimum. Increasing the trigger values to exclude noise can hide non-target cells from the sorting algorithm. This leads to lower purity.

Adjusting backscatter (BSC) and side scatter (SSC) gains

1. Scan and insert a cartridge containing the sample to be analyzed in the MACSQuant Tyto Instrument.
2. To start acquisition, click the **Start measurement** button in the progress bar. As events begin to accumulate, open the **Channels** tab.
3. Adjust the gains for BSC and (optional) SSC to visualize the population of events in the dot plots appropriately. The signal intensities in the backscatter blue (BSB), backscatter violet (BSV), and backscatter red (BSR) channel should be correlated by adjusting the voltage gains for the BSC channels.

Setting the noise threshold

The noise threshold setting is based on BSB, BSR, and BSV signals. Signals below the threshold are ignored and not saved or considered for sort decisions. Adjust this setting to minimize noise and debris while maintaining detection of events of interest (**Figure 2**). Noise threshold adjustments affect all BSC channels equally. Events in the sort gate are automatically backgated in the noise threshold plots. The backgated events have the color of the corresponding sort gate.

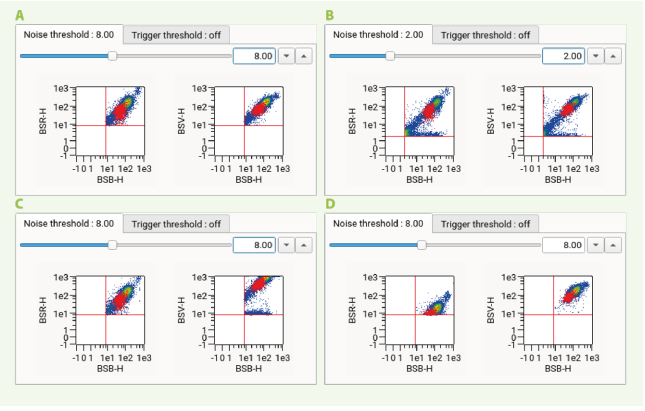


Figure 2: Example of different gain and threshold settings shown for white blood cells. Cells in the sort gate are automatically backgated (red). (A) Good settings for noise threshold and gains. (B) Noise threshold is set too low. Noise signals are disturbing the measurement. (C) BSV-H gain is set too high. High BSV-H signals are clipped. (D) BSR-H gain is set too low. The noise threshold cuts off events in the BSC channels.

To set the noise threshold:

1. Go to the **Channels** tab.
2. Go to the **Noise threshold** tab.
3. Click and hold the red threshold line in one of the BSC plots and move it to adjust the value.

Setting fluorescence channel gains

When setting the fluorescence channel gains, consider the following recommendations. Adjust the gain:

- if the negative or the positive population is not on scale.
- to optimize the signal-to-noise ratio.
- to maintain good signal resolution.
- so that the fluorescence intensity is brightest in its primary detection channel.
- Avoid gains above 700.
- Avoid adjustments that increase the background variability.

1. To view the first fluorescent parameter, format one of the dot plot displays.
2. In the **Channels** tab, adjust the gains for the specific fluorescence channel as needed to get both the positive and negative population on scale.

Setting the trigger threshold (optional)

The trigger threshold can be used as an additional trigger to discard irrelevant events. It can be set on a fluorescent or SSC channel. Signals below the threshold are not recorded or considered for sort decisions.

1. Go to the **Channels** tab.
2. Go to the **Trigger threshold** tab.
3. Select your channel.
4. Click and hold the red threshold line in one of the histograms and move it to adjust the value.
5. To activate the trigger threshold, select the checkbox.

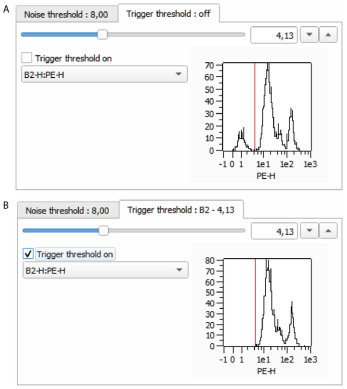


Figure 3: Optional trigger threshold deactivated (A) and activated (B)

Saving workspace files

1. Click the **Save** button on the toolbar and select **Workspace**.
2. The **Private** folder is automatically selected.
3. Enter a name for the new workspace file and click **Save**.

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, the Miltenyi Biotec logo, Tyto, the Tyto logo, Vio, VioBlue, and VioGreen are registered trademarks or trademarks of Miltenyi Biotec B.V. & Co. KG and/or its affiliates in various countries worldwide.
Copyright © 2024 Miltenyi Biotec and/or its affiliates. All rights reserved.

140-006-417-004.04