

How to use a MACSQuant® Instrument Express Mode in Custom Login

Express Modes

1. Introduction

Express Modes

The MACSQuantify™ Software includes a number of data acquisition and analysis tools that have been developed for an optimized and automated measurement of cells stained with Miltenyi Biotec reagent kits, e.g. MACS® Control Antibody Cocktails or Enrichment and Enumeration Kits. These Express Modes simplify flow cytometric analysis via predefined experiment settings as well as acquisition and analysis templates. Moreover, Express Modes apply an automated gating strategy to your cells of interest that will be automatically adjusted for each data file individually to achieve optimal results.

MACSQuantify Software login

MACSQuantify Software provides two user interfaces that you can use for your flow cytometric analysis:

- Express Login
- Custom Login

Both logins allow users to perform complex analyses fast and independently by providing Express Modes for predefined experiments. The Express Login provides a simplified interface and has been designed for use with predefined analysis templates. The Custom Login is designed to fit the needs of advanced users. In this mode, the user can change any settings of an Express Mode to adjust it to user defined conditions.

Sample grouping function in MACSQuantify Software

The sample grouping function in MACSQuantify Software allows the user to save more than one sample within one data file. For Express Modes, this is relevant for protocols that require a gate to be applied from one sample to another. In Express Modes that use a control staining sample it is mandatory to group the samples.

Note: When not using the Express Modes, the grouping function can be also used for combining two or more samples, for example, when measuring volumes larger than 450 µL.

Grouped samples can be ungrouped after acquisition and analyzed individually if desired. Ungrouped samples can be analyzed manually, however they cannot be analyzed by an Express Mode that requires grouped samples.

2. Execution of Express Modes not using grouped samples

1. Log into software.

Use the Custom Login. Make sure the instrument is in acquisition mode (bottles are illuminated and system status displays "Acquisition mode").

For optimal results of the Express Modes it is important to perform recommended calibration and compensation of the instrument.

2. Load instrument settings.

Click the **Open** button or use the menu bar (file → open) to open an instrument settings file. Choose an applicable setting for the measurement from the **Instrument settings** tab (see fig. 1). For the creation of an instrument settings file please refer to the MACSQuant Instrument user manual.

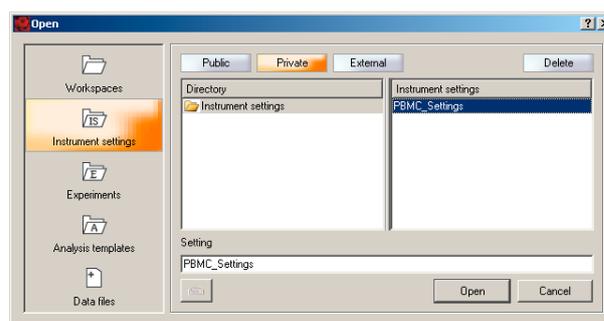
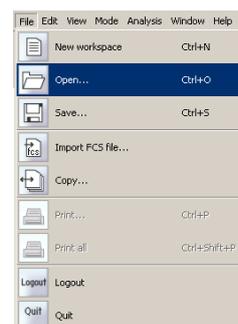


Figure 1: Loading of instrument settings

(Optional) Check and set the trigger to the correct channel (for requirements refer to the according data sheet). For example, MACS® Control stained samples will be measured with a CD45-VioBlue® trigger, whereas samples stained with other kits (e.g. EPC Enrichment and Enumeration Kit, human) will be measured with a FSC trigger. To select the channel for the trigger, click the **Channels** tab (see fig. 2). Consult with the data sheet for trigger requirements.

Note: For instrument settings requirements, refer to the relevant MACS Control Cocktail or kit data sheet. When using a Chill Rack, only select one single instrument setting. Express Modes are optimized for measurements with hlog axis settings. Refer to the MACSQuant Instrument user manual for information on setting axes.

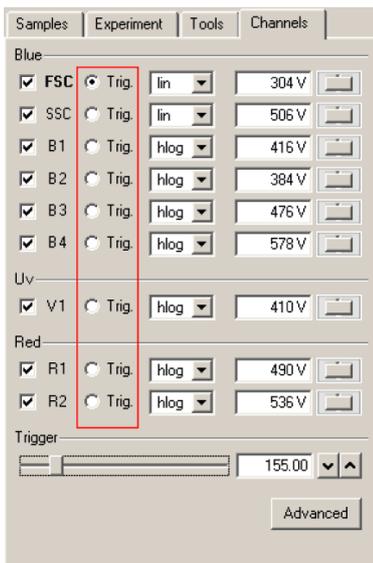


Figure 2: Select the channel for the trigger to be used by checking the trigger button.

3. Select sample rack.

Select the rack type from the **Rack** pull-down menu in the **Experiment** tab (fig. 3). For Chill Racks, define the rack positions that contain your samples by marking them with a mouse click.

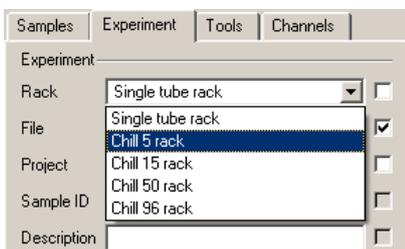


Figure 3: Selection of rack type.

4. Select an Express Mode.

In the **Settings** tab, click on the **Express** button, then select **Analysis** from the **Type** pull-down menu and chose your desired Express Mode from the **Mode** pull-down list (see fig. 4). By the selection of the Express Mode all experiment settings are loaded automatically. In Custom Login the loaded values are shown in the respective fields in the **Experiment** tab.

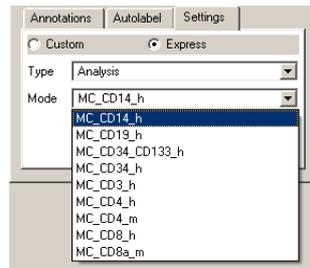


Figure 4: Selection of Express Mode.

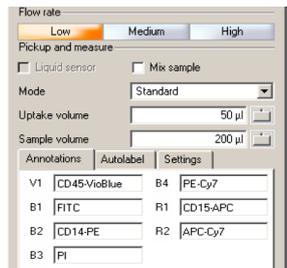


Figure 5: Loaded parameters of the Express Mode MC_CD14_h.

5. Enter the **Sample ID** and **Description**.

Use the **Sample ID** and **Description** fields to enter relevant sample information.



6. (Optional) Changing of predefined experiment settings.

The Custom Login allows you to change the default experiment settings defined by the Express Mode. This might be necessary in cases where the recommended protocol has been changed, e.g., sample volume has been changed from the kit recommendations (see fig. 6).

Note: Changes to the Express Mode settings can affect the analysis calculations. For example, if the sample uptake volume is reduced, there may not be enough events in the sample for an accurate analysis.

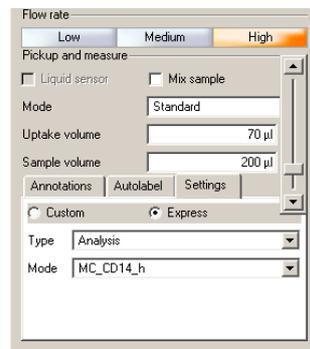


Figure 6: Change of default settings, e.g., flow rate and uptake volume.

7. Start the measurement.

Click the **Start Measurement** button (▶) to start acquisition and analysis. The acquisition page defined by the selected Express Mode will appear on the screen after acquisition starts. After acquisition the Express Mode will proceed automatically to the analysis. When measuring with the single tube rack the analysis page is shown on the screen until a new measurement is started. All relevant information (acquisition page, analysis page, type of Express Mode) are saved within the data file and do not have to be saved separately.

8. Backup data files.

Click the **Backup** button (📁) to backup data files. For detailed data backup information please refer to the MACSQuant Instrument user manual.

9. For analysis of Express Mode data on a separate PC proceed with 4. Analysis of Express Mode data files on computer.

3. Execution of Express Modes using grouped samples

1. Log into software.

Use the Custom Login. Make sure the instrument is in acquisition mode (bottles are illuminated and system status displays "Acquisition mode").

For optimal results of the Express Modes it is important to perform recommended calibration and compensation of the instrument.

2. Load instrument settings.

Click the **Open** button or use the menu bar (File → Open) to open an instrument settings file. Choose an applicable setting for the measurement from the **Instrument settings** tab. For the creation of an instrument settings file please refer to the MACSQuant Instrument user manual (see fig. 1). Check and set the trigger to the correct channel (for requirements refer to the according data sheet). For example, MACS Control Cocktail stained samples will be measured with a CD45-VioBlue trigger, whereas samples stained with other MACS Assay (e.g. EPC Enrichment and Enumeration Kit, human) will be measured with a FSC trigger. To select the channel for the trigger, click the **Channels** tab in Custom Login (see fig.2). Consult with the data sheet for trigger requirements.

Note: For instrument settings requirements, refer to the relevant MACS Control Cocktail or kit data sheet. Using a Chill Rack you can only select one single instrument setting. Express Modes are optimized for measurements with hlog axis settings. Refer to the MACSQuant Instrument user manual for information on setting axes.

3. Select sample rack.

Select the rack type from the **Rack** pull-down menu in the **Experiment** tab (see fig. 3).

For an Express Mode using grouped samples Chill Racks must be selected.

4. Define sample positions.

Mark the rack positions that contain your samples by clicking on them. Group the desired sample positions by using the **group** button. Grouped samples will be assigned the same group number (see fig. 7).

Note: Do not group more positions than sample IDs available (see information in corresponding kit data sheet). Only rack positions that are adjacent and in columns can be grouped.

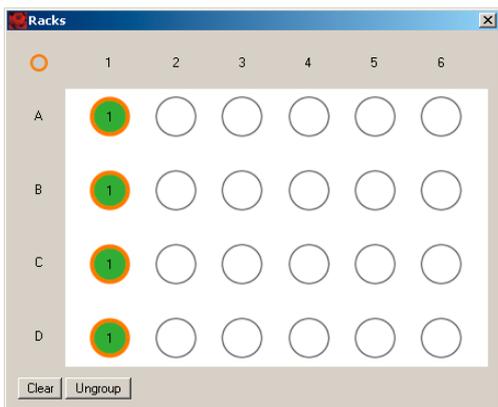


Figure 7: Rack positions A1 – D1 have been assigned to group 1 by using the group function.

5. Select an Express Mode.

In the **Settings** tab select **Analysis** from the **Type** pull-down menu and choose your desired Express Mode from the **Mode** pull-down list (see fig. 4). By the selection of the Express Mode all experiment settings are loaded automatically. In Custom Login the loaded values are shown in the respective fields of the **Experiment** tab. (see fig. 5).

6. Fill in sample information.

The sample IDs are applied on the rack positions according to the predefined order in the **Sample ID** pull-down menu (see fig. 8). To enter additional user defined information, use the description field. Please note that only one description may be assigned per group of samples.

7. (Optional) Changing of predefined experiment settings.

The Custom Login allows you to change the default experiment settings defined by the Express Mode (see fig. 6). For example, the sample IDs that were applied in the default order may be switched to reflect the sample positions in the Chill Rack. To change a sample ID, activate the sample well in the rack view and choose a **Sample ID** from the pull-down menu (see fig. 8). Avoid double assignments of sample IDs within a group of samples as this interferes with the subsequent analysis. Some Express Modes require the selection of mandatory sample IDs, e.g. "Control" in the Express Mode MC_CD34_CD133_h.

Note: Changes to the Express Mode settings can affect the analysis calculations. For example, if the sample uptake volume is reduced there may not be enough events in the sample for an accurate analysis.

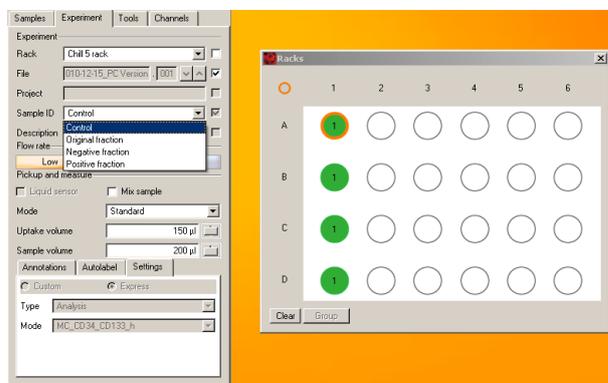


Figure 8: In order to change the default sample IDs activate the sample well and choose a sample ID from the pull-down menu.

8. Start the measurement.

Click the **Start Measurement** button (▶) to start acquisition and analysis. The acquisition page defined by the selected Express Mode will appear on the screen after acquisition starts. After acquisition the Express Mode will proceed automatically to the analysis. The analysis page is shown on the screen until a new measurement is started. All relevant information (acquisition page, analysis page, type of Express Mode) are saved within the data file and do not have to be saved separately.

9. Backup data files.

Click the **Backup** button (📁) to backup data files. For detailed data backup information please refer to the MACSQuant Instrument user manual.

10. For analysis of Express Mode data with a PC proceed with 4. Analysis of Express Mode data files on computer.

4. Analysis of Express Mode data files on computer

1. Analysis of data files in Custom Login.

Open the data file and call up its submenu by right clicking on the file name. Select **View with express**. **Analysis.<name of the Express Mode>** for accessing the analysis template. The analysis will be displayed on the screen.

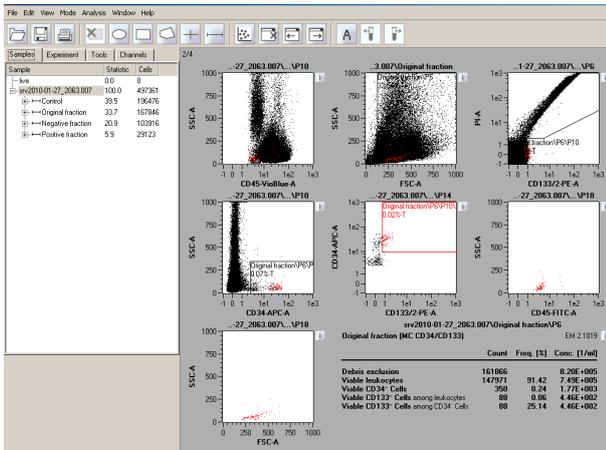
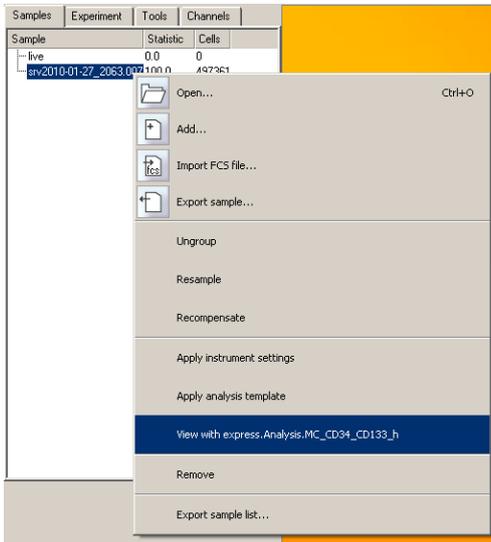
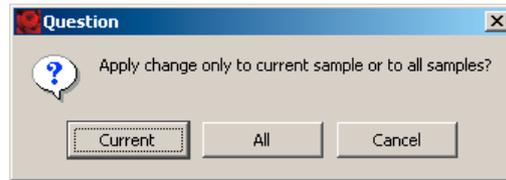


Figure 9: Excerpt from an analysis screenshot in Custom Mode user interface with the MC_CD34_CD133_h Express Mode.

In case you would like to change a gated region after analysis of the Express Mode data file, you can drag and drop it in the dot plot. In case of a grouped data file that links the regions within the single files a pop up window will ask you whether you want to apply the change only to the current single sample or to all grouped samples.

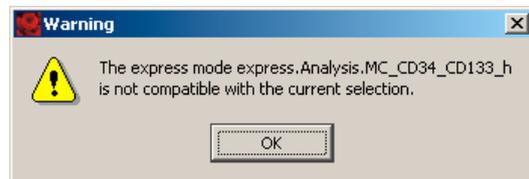
If adjustments to the gated regions are required, ensure that the **A** analysis button is not activated.



Example: You move a region on **Original fraction** (in MC_CD34_CD133_h Express Mode data file) and you click on **Current**, the region move only takes place on **Original fraction**. The corresponding linked regions on **Control**, **Negative fraction**, and **Positive fraction** are unaffected. Moreover, the moved region on **Original fraction** is unlinked from the other regions. When you click **All** the region move will be applied to the corresponding regions of all grouped samples within this data file.

5. Troubleshooting

1. Failure during Express Mode definition for acquisition.

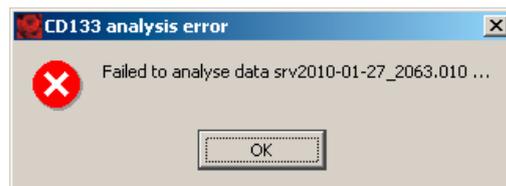


Warning window will appear when selecting an Express Mode for grouped samples in case of:

- no Chill Rack selected,
- sample positions not grouped,
- more sample positions grouped than sample IDs available.

Note: Sample positions that are not adjacent in columns or rows cannot be grouped.

2. Failure of analysis.



Warning window will appear after measurement in case of:

- double assignment of sample IDs in grouped Express Mode data files,
- no (or very few) events measured,
- not enough events measured in rare cell Express Modes (for detailed information see according data sheet).
- MACSQuantify Software on PC is set to the wrong instrument configuration. Go to **Edit -> Configuration** to select the configuration that was used to acquire the data file.