



Data acquisition and analysis without the MACSQuant® Analyzer 10

## General instructions for MACSPlex miRNA Kit – Cancer

### Data acquisition and analysis of the MACSPlex miRNA Kit – Cancer with another instrument than the MACSQuant® Analyzer 10

MACSPlex miRNA Kits are designed for detection of microRNAs (miRNAs). A prerequisite for the performance of MACSPlex Assays is a flow cytometer with a blue (e.g., 488 nm) and a red (e.g., 635 nm) laser and the ability to detect FITC, PE, and APC. For detailed information on how to run MACSPlex Assays on the MACSQuant Analyzer 10, please refer to the special protocol "Data acquisition and analysis of MACSPlex miRNA Kits using the MACSQuant® Analyzer Express Modes" available at the respective product page at [www.miltenyibiotec.com](http://www.miltenyibiotec.com). The instructions below apply for the use of other flow cytometers featuring the required optical configuration.

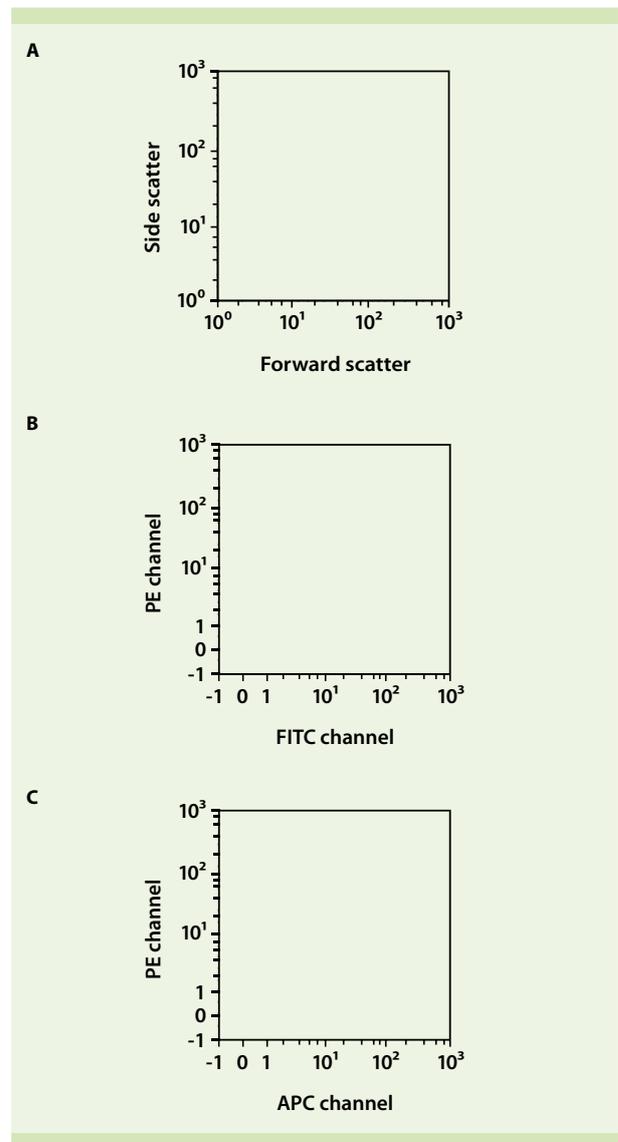
### General setup procedure

In order to setup flow cytometers MACSPlex Setup Beads 1 and Setup Beads 2 are provided in the kit.

1. Start the flow cytometer according to the manufacturer's instructions.
2. Open a new protocol.
3. Create three dot plots:
  - Forward scatter versus side scatter – dot plot
  - FITC (525 nm) versus PE (585 nm) – dot plot
  - PE versus APC (655 nm) – dot plot
4. Set all scatter axes to log scale and all fluorescence axes to hyperlog scale.

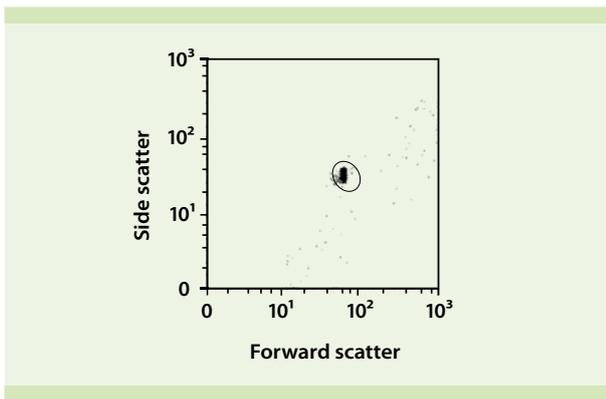
**Note:** The dot plots in the following example include hyperlog scales for all fluorescence axes. However, if this option is not available on your flow cytometer, log scales are also applicable.

5. Set all compensation to zero.



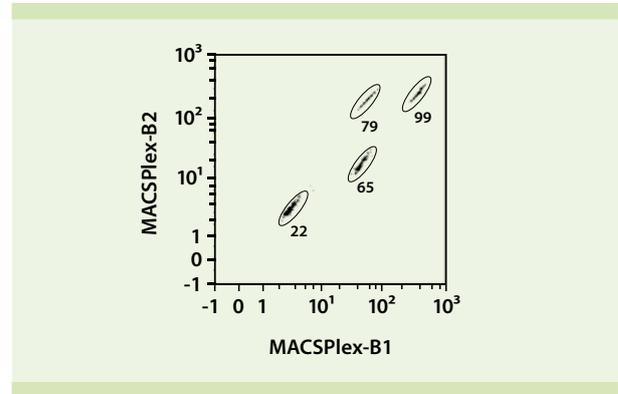
**Figure 1:** Analysis template, comprising three dot plots (A, B, and C).

6. Resuspend the MACSPlex Setup Beads 1 by vortexing for at least 30 seconds before use.
7. Pipette 200  $\mu$ L MACSPlex miRNA Setup Beads 1 into a tube or plate to be used on your flow cytometer.
8. Run the MACSPlex Setup Bead sample in the setup mode.  
If not available, choose a similar mode that allows the adjustment of all required parameters while running the MACSPlex Setup Bead 1 sample.
9. Adjust the forward scatter (FSC) and side scatter (SSC) PMT voltages and trigger or threshold (if applicable), so that the bead population is visible in the respective FSC/SSC dot plot.
10. Place a gate around singlets.
11. Activate the singlet gate in the FITC channel versus PE channel dot plot.
12. Adjust PMT voltages for the FITC and PE channels.  
Ensure that four bead populations are visible and placed within the plot.
13. Place four gates around the bead populations.
14. For each of the four gates activate depending APC versus PE gates.



**Figure 2:** Forward scatter versus side scatter dot plot of the MACSPlex Setup Beads 1 showing the singlet gate.

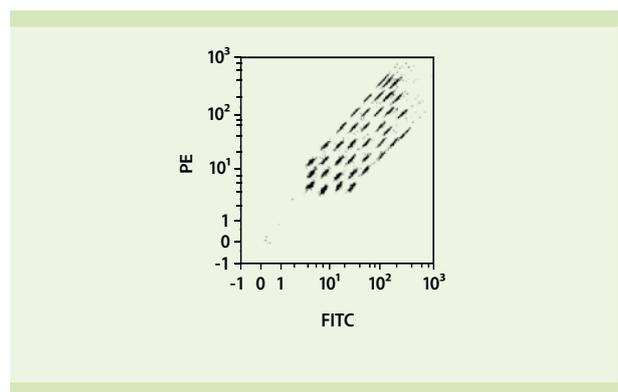
15. Ensure that the APC signal of the gated bead population shows a typical negative population peak (e.g., similar to unlabeled cells) for bead population 22, 65, and 99 (see figure 3 for naming of bead populations). Bead population 79 should give a clear signal above background. If this is not the case, adjust the PMT voltage of the APC channel accordingly.



**Figure 3:** FITC channel versus PE channel dot plot of MACSPlex Setup Beads 1 showing the subordinate gates, containing only singlets.

16. Resuspend the MACSPlex miRNA Setup Beads 2 by vortexing for at least 30 seconds before use.
17. Pipette 200  $\mu$ L MACSPlex miRNA Setup Beads 2 into a tube or plate to be used on your flow cytometer.
18. Ensure that 39 bead populations are visible and are placed within the PE versus FITC plot. Adjust PMT if necessary.
19. Place the remaining 35 gates around the bead populations.
20. Save the instrument settings.

There is no compensation required. Please proceed directly to data acquisition.



**Figure 4:** FITC channel versus PE channel dot plot of MACSPlex Setup Beads 2 showing the subordinate gates, containing only singlets.

## General acquisition procedure

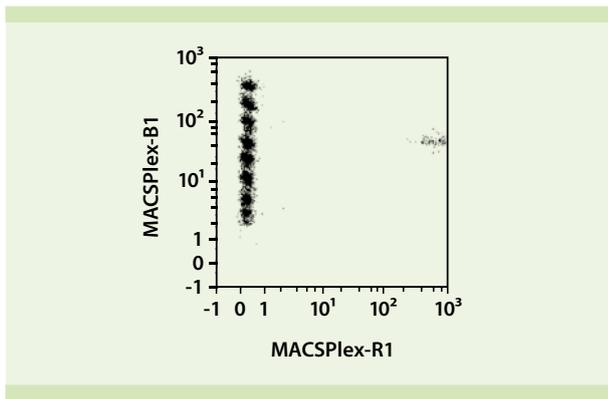
1. Change into acquisition mode.
2. Set the uptake volume to 50  $\mu\text{L}$  (of your 80  $\mu\text{L}$  sample).
3. Start the measurement of your samples.

**Note:** Do not change any settings during measurement of the standards or samples.

## General data analysis procedure

Load your acquisition files into a suitable analysis software. The following steps have to be applied to every sample.

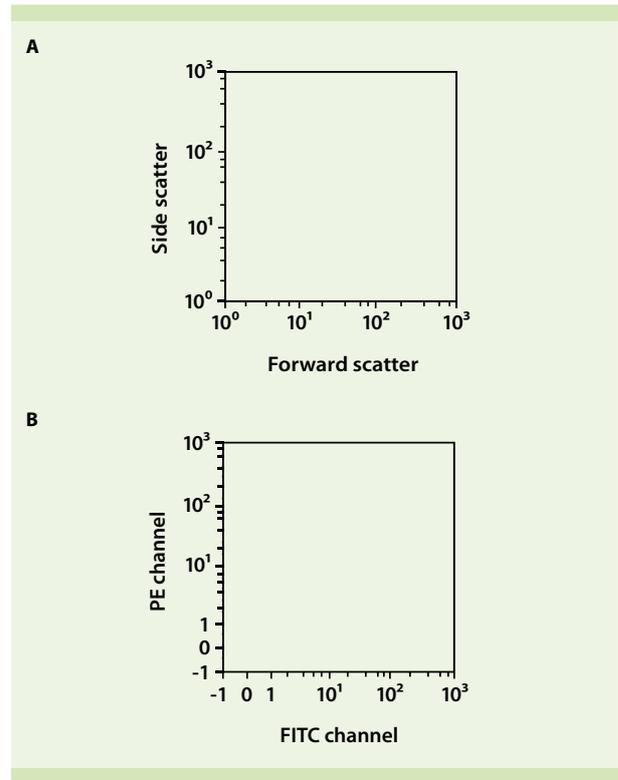
1. Open a new protocol.
2. Create two dot plots:
  - Forward scatter versus side scatter
  - FITC channel (525 nm) versus PE channel (585 nm)
3. Set all scatter axes to log scale and all fluorescence axes to hyperlog scale.
4. Place a gate around the singlets in the forward scatter versus side scatter dot plot.



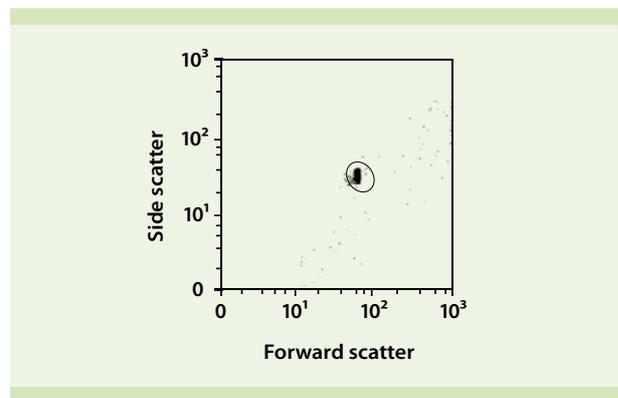
**Figure 5:** PE channel versus APC channel dot plot of MACSPlex Setup Beads 2. All bead populations besides Pop65 (positive control for APC channel) shows a typical negative signal.

**Note:** The 39 bead populations cannot be resolved by the PE signal intensity. Therefore, populations differing in the FITC channel appear as one population in this plot.

5. Activate the singlet gate (P1) in the FITC channel versus PE channel dot plot.



**Figure 6:** Analysis template containing a forward scatter versus side scatter (A) and a FITC channel versus PE channel (B) dot plot.



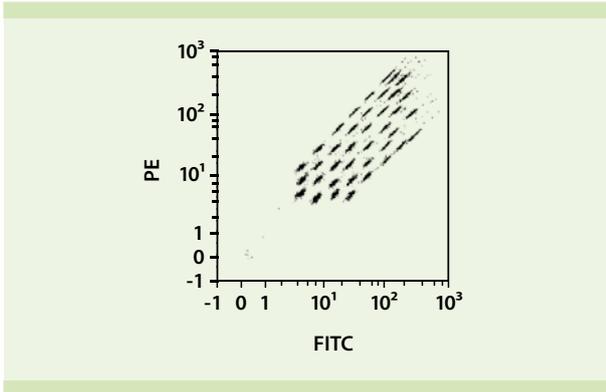
**Figure 7:** Forward scatter versus side scatter dot plot of MACSPlex miRNA Beads showing the singlet gate (P1).

6. Place a gate around every MACSPlex miRNA Bead populations.

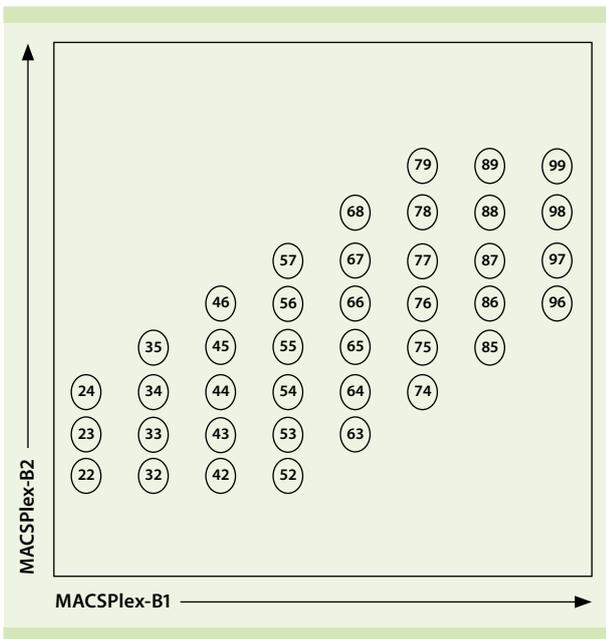
7. Create a statistics table displaying the APC medians for each MACSplex miRNA Bead population. Apply the same coding of MACSplex miRNA Bead populations as shown in figure 8.

**Note:** Your results will be more reliable, if your analysis software allows you to select the APC median or geometric mean instead of the mean.

**Note:** The appearance of the dot plots might vary depending on the used flow cytometer and analysis software. However, the overall arrangement of the MACSplex miRNA Bead populations should be similar to the example shown here.



**Figure 8:** Example of a FITC channel versus PE channel dot plot of the MACSplex miRNA Bead populations with activated singlet gate using the MACSplex miRNA Kit – Cancer, human.



**Figure 9:** Detection of MACSplex miRNA Cancer Bead populations in a MACSplex-B1 (FITC) versus MACSplex-B2 (PE) dot plot.

## Calculation of concentrations

For calculation details refer to MACSplex miRNA Kit – Cancer, human data sheet.

For further information, please contact Miltenyi Biotec Technical Support at [www.miltenyibiotec.com](http://www.miltenyibiotec.com)



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