# Miltenyi Biotec

# General instructions for MACSPlex Assay
# Data acquisition and analysis

## General instructions for MACSPlex Assay data acquisition and analysis

MACSPlex Assays are designed for determining concentrations of soluble analytes in a single sample. A prerequisite for the performance of MACSPlex Assays is a flow cytometer with blue (e.g., 488 nm) and red (e.g., 635 nm) lasers and the capability to detect FITC, PE, and APC.
For detailed information on the principle of MACSPlex Assays and its performance using MACSQuant® Analyzer and MACSQuant Analyzer 10, please refer to the respective data sheet.
The instructions below apply for the use of other flow cytometers featuring the required optical configuration.

## General setup procedure

For the setup of flow cytometers other than the MACSQuant Analyzer and MACSQuant Analyzer 10, the MACSPlex Kits provide MACSPlex (MPx) Setup Beads.
1. Start the flow cytometer according to the manufacturer's instructions.
2. Open a new protocol.
3. Create two dot plots and one histogram:
   • Forward scatter versus side scatter – dot plot
   • FITC (525 nm) versus PE (585 nm) – dot plot
   • APC (655 nm) – histogram
4. Set all scatter axes to log scale and all fluorescence axes to hlog scale.

**Note:** The dot plots in the following example include hlog scales. However, if this option is not available for the flow cytometer of choice, log scales are also applicable.
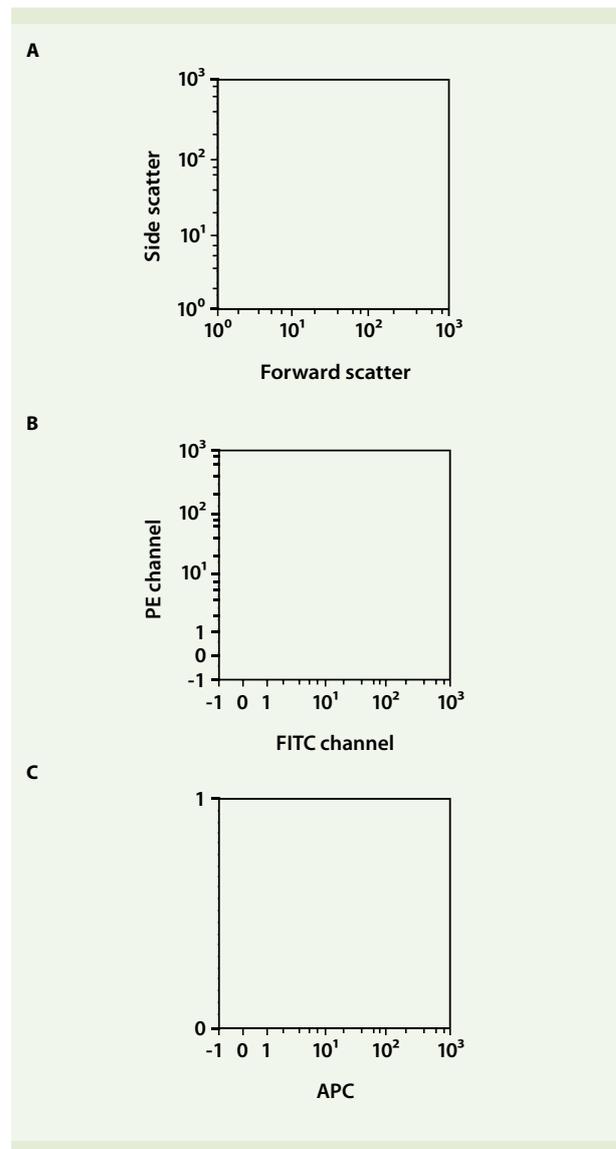


**Figure 1:** Analysis template, including two dot plots (A and B) and one histogram (C).

5. Set all compensation to zero.
6. Resuspend the MPx Setup Beads by vortexing for at least 30 seconds before use.
7. Dilute MPx Setup Beads by dispensing one drop in 1 mL of MACSPlex buffer.
8. Run MPx Setup Bead sample in setup mode. If not available, choose a similar mode that allows the adjustment of all required parameters while running the MPx Setup Bead 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.
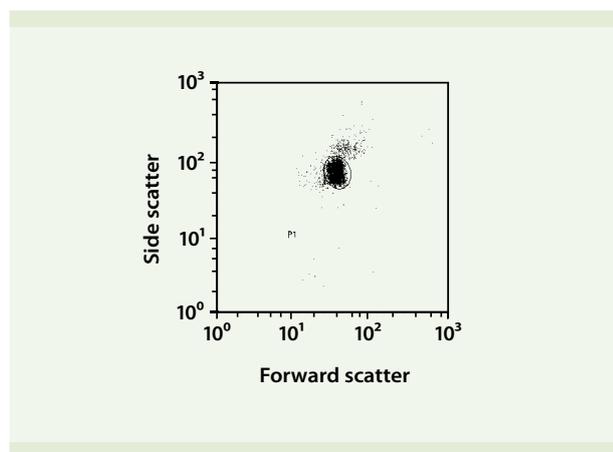10. Place a gate around singlets.



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

11. Activate the singlet gate in the FITC channel versus PE channel dot plot.
12. Adjust PMT voltages for the FITC and PE channels, ensuring that the bead population is placed in the center of the plot.
13. Place a gate around the bead population.
14. Activate gate P2, containing only MPx Setup Bead singlets, in the APC histogram.
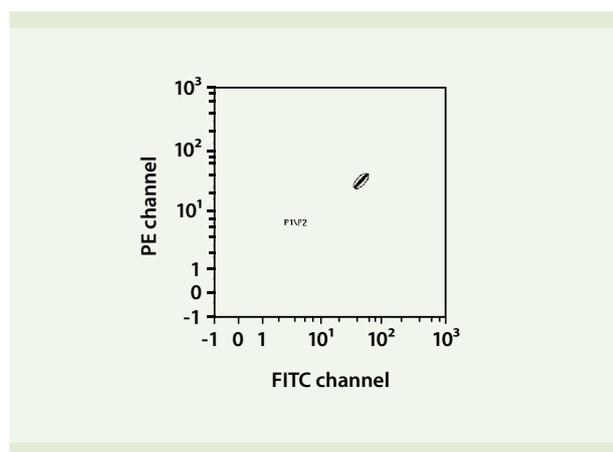


**Figure 3:** FITC channel versus PE channel dot plot of MPx Setup Beads showing the subordinate gate P2, containing only singlets.

15. Ensure that the APC signal of the gated bead population shows a typical negative population peak (e.g., similar to unlabeled cells). If this is not the case, adjust the PMT voltage of the APC channel accordingly.
16. Save the instrument settings.

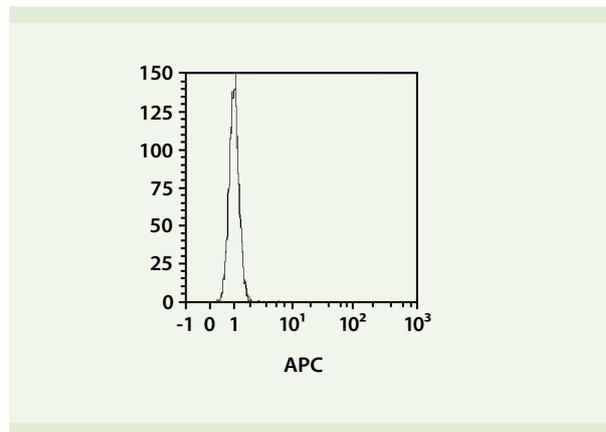There is no compensation required. Please proceed directly to data acquisition.



**Figure 4:** APC histogram of MPx Setup Beads with the activated subordinate gate P2, containing all MPx Setup Bead singlets.

## General acquisition procedure

1. Change into acquisition mode.
2. Set the instrument to stop the measurement when 300 events per analyte have been aquired in the singlet gate (P1).
3. Delete the gate P2, containing all MPx Setup Bead singlets in the FITC channel versus PE channel dot plot.
4. Always start the measurement with the MPx Standards, , e.g., MPx Cytokine 12 Standard, beginning with 0 pg/mL and finishing with the top standard. Process samples afterwards.

**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 of the MPx Standard dilution and every unknown sample measured. Begin the data analysis with MPx Standard dilutions before proceeding with samples.

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 hlog scale.

**Note:** The dot plots in the following example include hyperlog scales. However, if this option is not available for the flow cytometer of choice, log scales are also applicable.
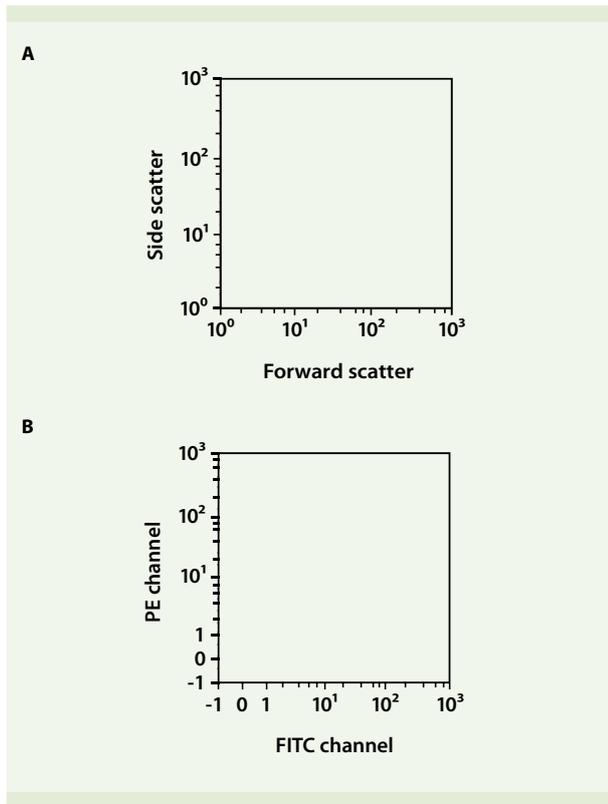
**Figure 5:** Analysis template containing a forward scatter versus side scatter (A) and a FITC channel versus PE channel (B) dot plot.

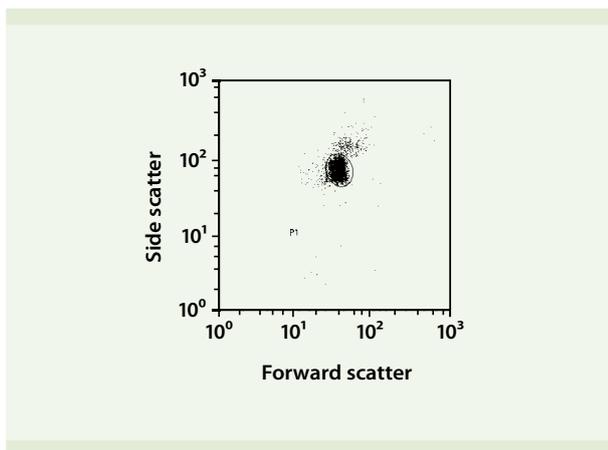4. Place a gate around the singlets in the forward scatter versus side scatter dot plot.

**Figure 6:** Forward scatter versus side scatter dot plot of MPx Capture Beads showing the singlet gate (P1).

5. Activate the singlet gate (P1) in the FITC channel versus PE channel dot plot.
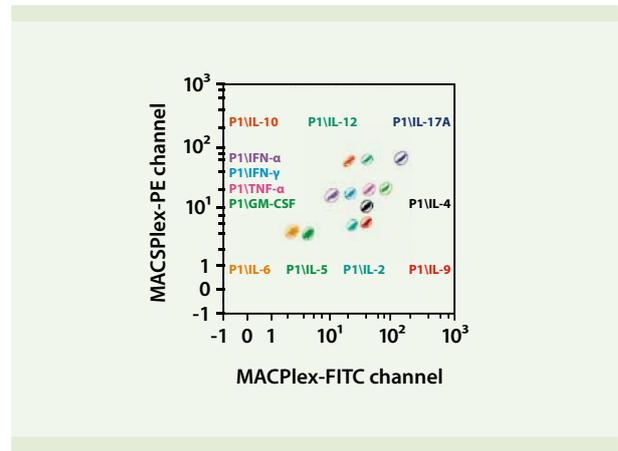6. Place a gate around every MPx Capture Bead population.

**Figure 7:** Example of a FITC channel versus PE channel dot plot of the MPx Capture Bead populations with activated singlet gate using the MACSPlex Cytokine 12 Kit, human. The appearance of the dot plots might vary depending on the used flow cytometer and analysis software. However, the overall arrangement of the MPx Capture Bead populations should be similar to the example shown here.

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

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

| Region | APC-A Median |
|---|---|
| IL-10 | 2.64 |
| IL-12 | 2.78 |
| IL-17A | 2.43 |
| IFN-alpha | 1.18 |
| IFN-gamma | 1.55 |
| TNF-alpha | 1.25 |
| GM-CSF | 1.12 |
| IL-4 | 0.93 |
| IL-6 | 0.78 |
| IL-5 | 0.70 |
| IL-2 | 1.25 |
| IL-9 | 1.03 |

**Figure 8:** Statistics table showing the APC medians for each MPx Capture Bead population.

# Calculation of analyte concentrations

1. After applying the analysis protocol to all acquired MPx Standard dilutions and samples, transfer the APC medians into a spreadsheet of an applicable software.

2. Create a standard curve for every measured analyte using the APC medians of the MPx Standard dilutions.

3. Calculate the concentration of each measured analyte in the samples using the respective standard curve.
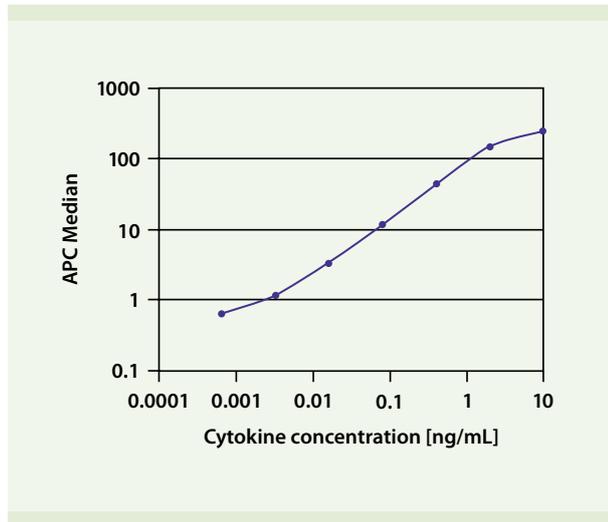


**Figure 9:** Standard curve of the measured analyte using the APC medians of MPx Standard dilutions.

For further information, please contact Miltenyi Biotec Technical Support at **www.miltenyibiotec.com**

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