Miltenyi Biotec

MACSPlex Exosome Kit

General instructions

Data acquisition and analysis of the MACSPlex Exosome Kit with the MACSQuant® Analyzer 10 or other instruments

The MACSPlex Exosome Kit is designed for determining exosome surface markers. 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. The kit comprise a cocktail of various fluorescently labeled bead populations, each coated with a specific antibody binding the respective surface epitope. The 39 bead populations can be distinguished by different fluorescence intensities by flow cytometry. 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 Exosome Setup Beads 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 (fig. 1):
   - 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.

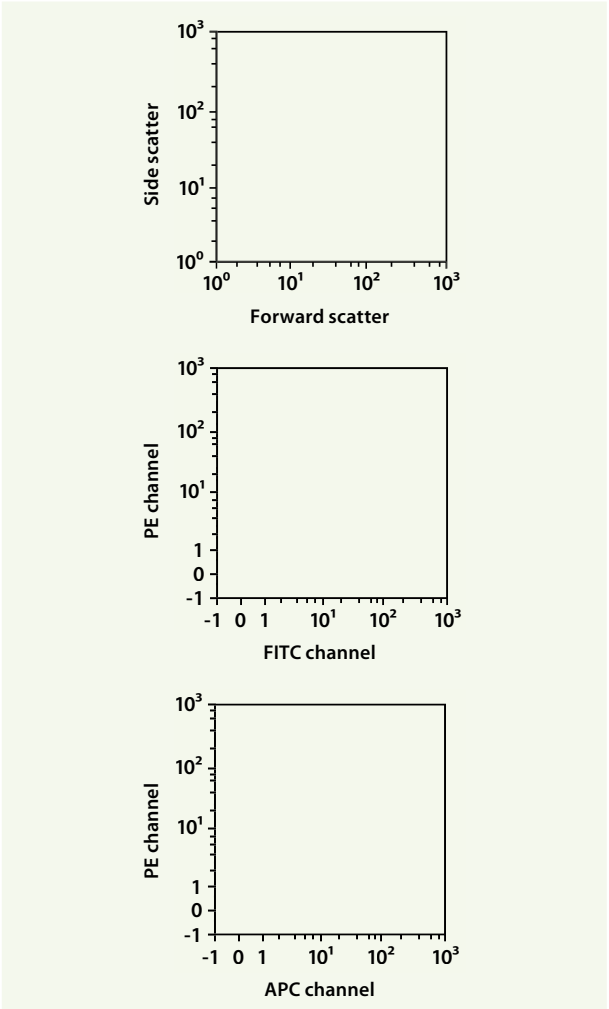

Figure 1: How to set up the analysis template. It comprises three dot plots.

5. Set all compensation to zero.
6. Resuspend the MACSPlex Exosome Setup Beads by vortexing for at least 30 seconds before use.
7. Pipette 150 µL MACSPlex Exosome Setup Beads into a tube or plate to be used on your flow cytometer.
8. Run the MACSPlex Exosome 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 Exosome 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 (fig. 2).
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 39 bead populations are visible and placed within the plot (fig. 3).
13. Place 39 gates around the bead populations (P1/P2–P1/P40).
14. For each of the 39 gates activate depending APC versus PE gates.

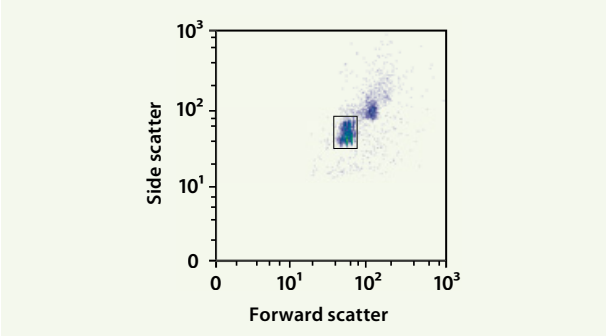

Figure 2: Forward scatter versus side scatter dot plot of the MACSPlex Exosome Setup Beads showing the singlet gate.

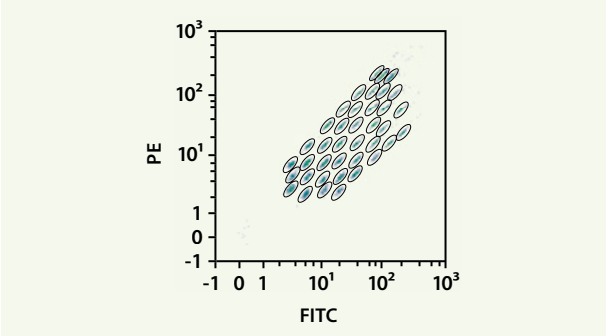

Figure 3: FITC channel versus PE channel dot plot of MACSPlex Exosome Setup Beads. The subordinate gate P1/P2–P1/P40, containing only singlets.

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 (fig. 4).

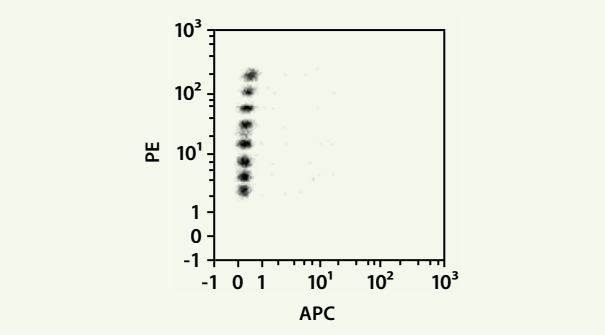

Figure 4: PE channel versus APC channel dot plot displaying the signal of single bead types.

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

16. Save the instrument settings.

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

General acquisition procedure

1. Change into acquisition mode.
2. Start the measurement of your samples.

Note: Do not change any settings during measurement of the 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 (fig. 5):
   - Forward scatter versus side scatter
   - FITC channel (525 nm) versus PE channel (585 nm)

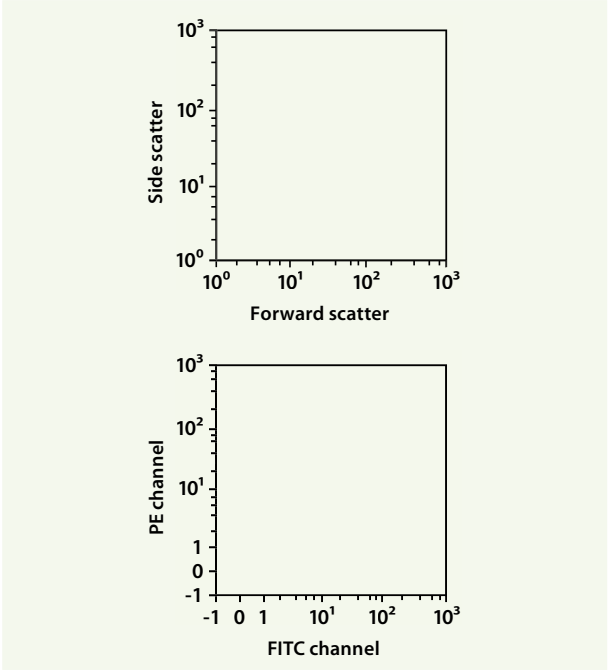

Figure 5: Template. It contains a forward scatter versus side scatter (A) and a FITC channel versus PE channel (B) dot plot.

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 (fig. 6).
5. Activate the singlet gate (P1) in the FITC channel versus PE channel dot plot.
6. Place a gate around every MACSPlex Exosome Capture Bead population (fig. 3).
7. Using the same coding of MACSPlex Exosome Capture Bead populations as shown in figure 7, create a statistics table displaying the APC medians for each MACSPlex Exosome Capture Bead populations (fig. 8).

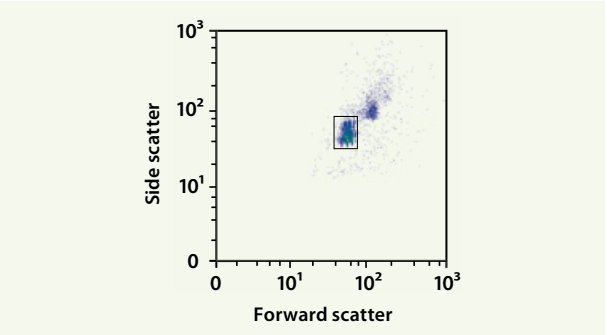

Figure 6: Forward scatter versus side scatter dot plot of MACSPlex Exosome Capture Beads. The singlet gate (P1).

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 Exosome Capture Bead populations should be similar to the example shown here.

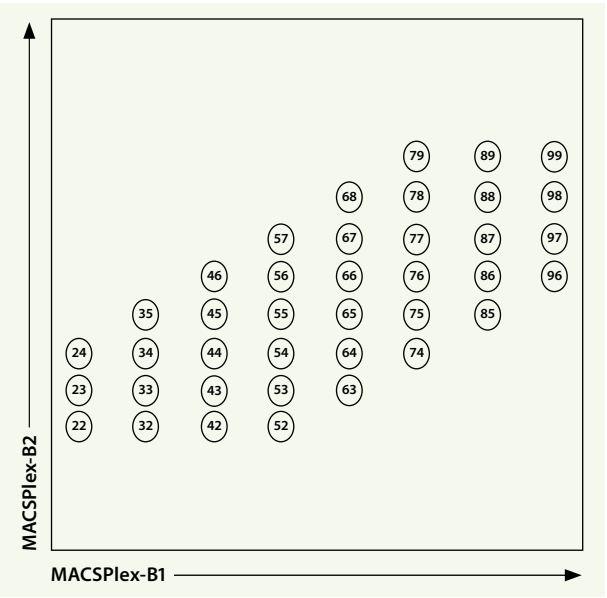

Figure 7: Detection of MACSPlex Exosome Capture Bead populations. MACSPlex-B1 (FITC) versus MACSPlex-B2 (PE) dot plot.

No.	Median
22	10.38
24	2.51
32	3.19
33	484.23
34	29.61
35	73.93
42	4.73
43	3.42
44	8.86
45	20.19
46	6.20
52	3.81
53	676.49
54	218.46
55	38.15
56	2169.18
57	33.23
63	2.70
64	2.62
65	917.48
66	483.65
67	2370.60
68	21.67
74	2.69
75	66.04
76	4.00
77	2736.44
78	6.10
79	41.84
85	33.09
86	1.74
87	6.41
88	12.34
89	4.55
96	15.95
97	3.51
98	4.30
99	6.60

Table 1: Statistics table showing the APC medians for each MACSPlex Exosome Capture Bead population.

Calculations

For calculation details refer to MACSPlex Exosome Kit, human data sheet.

LEARN MORE

For further information, please contact Miltenyi Biotec Technical Support at

▸ **miltenyibiotec.com**

Miltenyi Biotec

Miltenyi Biotec B.V. & Co. KG | Friedrich-Ebert-Straße 68 | 51429 Bergisch Gladbach | Germany | Phone +49 2204 8306-0 | Fax +49 2204 85197
macsde@miltenyi.com | **www.miltenyibiotec.com**

Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

130-131-544.03