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Miltenyi Biotec

MACSPlex Exosome Kit

human

For up to 96 tests Order no. 130-108-813

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140-004-948.03

Content

1. Description

This product is for research use only.

Components

For up to 96 tests:

- 1.5 mL MACSPlex Exosome Capture Beads, human
- 0.5 mL MACSPlex Exosome Detection Reagent CD9, human
- 0.5 mL MACSPlex Exosome Detection Reagent CD63, human
- 0.5 mL MACSPlex Exosome Detection Reagent CD81, human
- 2×100 mL MACSPlex Buffer
- 1.5 mL MACSPlex Exosome Setup Beads
- 1 MACSPlex Filter Plate for 96 tests
- 2 adhesive foils for 96 tests

▲ Kit components should not be substituted or mixed with those from other kits or lots.

▲ MACSPlex Exosome Detection Reagents CD9, CD63, and CD81 could be combined to create a detection cocktail.

Size	Up to 96 tests
Product format	MACSPlex Exosome Capture Beads and MACSPlex Exosome Setup Beads are supplied in buffer containing stabilizer and 0.05% sodium azide. MACSPlex Buffer contains 0.08% sodium azide.
Storage	Store MACSPlex Exosome Capture Beads, MACSPlex Exosome Detection Reagents, and MACSPlex Exosome Setup Beads protected from light at 2–8 °C. Do not freeze. Store MACSPlex Buffer, MACSPlex Filter Plate, and adhesive foils at room temperature. The expiration dates are indicated on the vial labels.

1.1 Principle of MACSPlex Exosome Kits

The MACSPlex Exosome Kits allow detection of 37 exosomal surface epitopes plus two isotype controls. The MACSPlex Exosome Kit comprises 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 detected in the FITC and PE channel of flow cytometers (B1 and B2 channel of MACSQuant Analyzers).

Exosomes are incubated with the antibody-coated MACSPlex Exosome Capture Beads. Subsequently or in parallel, exosomes bound to the MACSPlex Exosome Capture Beads are labeled with the MACSPlex Exosome Detection Reagents. The MACSPlex Exosome Detection Reagents can also be combined to create a cocktail comprising of MACSPlex Exosome Detection Reagent for CD9, CD63, and CD81. Consequently, sandwich complexes are formed between the MACSPlex Exosome Capture Bead, exosome, and the detection reagent (fig. 1.1). These complexes can be analyzed based on the fluorescence characteristics of both the MACSPlex Exosome Capture Bead and the detection reagent. Positive signals indicate the presence of the respective surface epitope within the exosome population (fig. 1.2).

It is also possible to compare different exosome samples using the MACSPlex Exosome Kit allowing semi-quantitative analysis of differential surface epitopes.

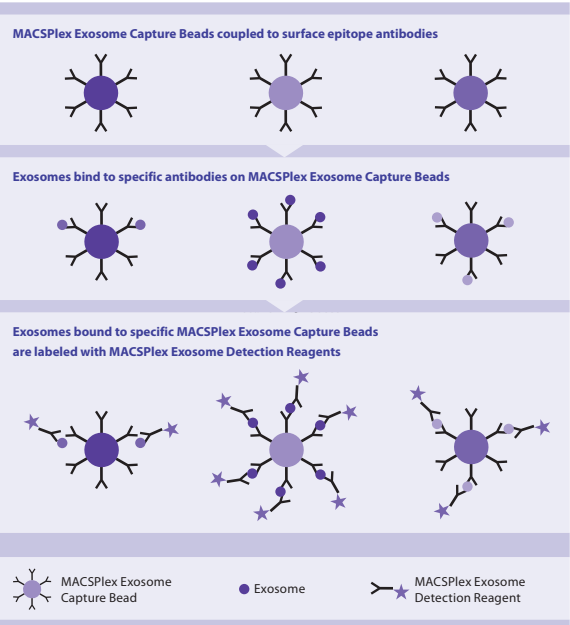

Figure 1.1: Principle of MACSPlex Exosome Kit.

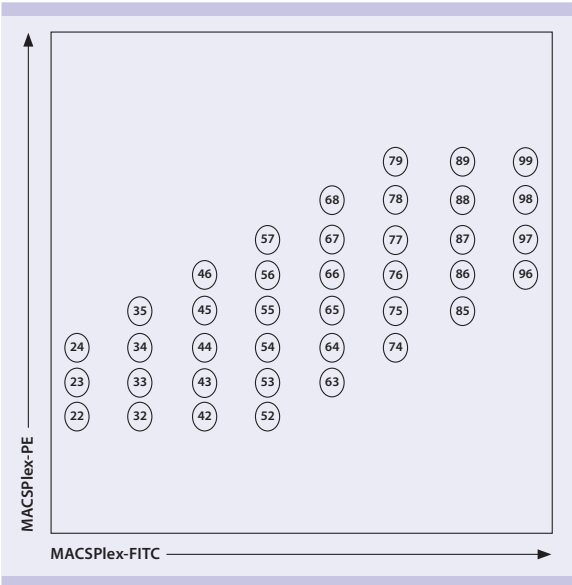

Figure 1.2: Detection of MACSPlex Exosome Capture Bead populations in a MACSPlex-FITC (B1) versus MACSPlex-PE (B2) dot plot.

1.2 MACSPlex Exosome Kit

The MACSPlex Exosome Kit can be performed in the delivered filter plates (only # 130-108-813) or as single reactions in tubes. Using the filter plate, the washing can be performed on a vacuum manifold or by using a centrifuge with an adapter for microtiter plates. In most instances, an one hour incubation time is sufficient to bind and stain exosomes on the MACSPlex Exosome Capture Beads. For samples comprising low amounts of exosomes like supernatants of some cell types, we recommend to prolong the incubation time to overnight to increase sensitivity.

▲ For the overnight protocols, staining is performed after the initial exosome binding to the MACSPlex Exosome Capture Beads.

▲ A negative control using only buffer is strongly recommended to determine non-specific signals. The negative control is subtracted from the sample signals to correct potential non-specific signals.

▲ For the filter plate a centrifuge with an adapter for microtiter plates can be used instead of a vacuum manifold: Put the MACSPlex Filter Plate on top of a conventional 96 flat–bottom microtiter plate without lid and place both into the adapter. Centrifuge at 300×g for 3 minutes at room temperature.

▲ **Note:** Do not overdry.

▲ It is recommended to use a cocktail of the three MACSPlex Exosome Detection Reagents CD9, CD63, and CD81 for a broad exosome staining. For each experiment, a mastermix could be set up using 5 µL of each MACSPlex Exosome Detection Reagent for each reaction, i.e., 15 µL MACSPlex Exosome Detection Reagent cocktail per well.

▲ **Note:** Storage of mastermixes is not recommended.

▲ To detect other surface proteins on the exosomes samples, APC-conjugated antibodies can be used instead of the MACSPlex Exosome Detection Reagent. The optimal amount of detection antibody should be titrated. It is recommended to use 5 µL with a concentration of 0.1 µg/µL or 0.5 µg APC-antibody per reaction.

1.3 Applications

The MACSPlex Exosome Kit has been developed for the simultaneous flow cytometric detection of 37 surface epitopes that are known to be present on different exosomes plus two isotype control beads (table 1).

1.4 Reagent and instrument requirements

- MACSQuant® X (# 130-105-100), MACSQuant Analyzer 10 (# 130-096-343), or other flow cytometers equipped with blue (488 nm) and red (635 nm) lasers able to discriminate FITC, PE, and APC fluorescence.

  ▲ **Note:** The MACSQuant VYB cannot be used.

- MACS® Chill 96 Rack (# 130-094-459), when using the MACSQuant Analyzer 10.
- MACSQuant Calibration Beads (# 130-093-607), when using the MACSQuant X or MACSQuant Analyzer 10.
- Disposable pipette tips
- (Optional) Exosome Isolation Kit CD9, human (# 130-110-913) or Exosome Isolation Kit CD63, human (# 130-110-918) or Exosome Isolation Kit CD81, human (# 130-110-914) for exosome pre-enrichment from plasma without ultracentrifugation.

Microtiter plate format

- Orbital shaker for 96-well plates (frequency 450 rpm).
- Vacuum manifold or centrifuge with adapters to accommodate microtiter plates.
- Multichannel pipettor is recommended.

Tube format

- MACSmix™ Tube Rotator (# 130-090-753) or an orbital shaker for tubes (450 rpm)

- Polypropylene or polystyrene reagent tubes
- 96-well round bottom plate

No.	Antibody	Isotype
22	CD3	mIgG2a
23	CD4	mIgG2a
24	CD19	mIgG1
32	CD8	mIgG2a
33	HLA-DRDPDQ	REA
34	CD56	REA
35	CD105	REA
42	CD2	mIgG2b
43	CD1c	mIgG2a
44	CD25	mIgG1
45	CD49e	mIgG2b
46	ROR1	mIgG1κ
52	CD209	mIgG1
53	CD9	mIgG1
54	SSEA-4	REA
55	HLA-ABC	REA
56	CD63	mIgG1κ
57	CD40	mIgG1κ
63	CD62P	REA
64	CD11c	mIgG2b

No.	Antibody	Isotype
65	CD81	REA
66	MCSP	mIgG1
67	CD146	mIgG1
68	CD41b	REA
74	CD42a	REA
75	CD24	mIgG1
76	CD86	mIgG1
77	CD44	mIgG1
78	CD326	mIgG1
79	CD133/1	mIgG1κ
85	CD29	mIgG1κ
86	CD69	mIgG1κ
87	CD142	mIgG1κ
88	CD45	mIgG2a
89	CD31	mIgG1
96	REA Control	REA
97	CD20	mIgG1
98	CD14	mIgG2a
99	mIgG1 control	mIgG1

Table 1.1: Overview of surface marker antibodies used for the MACSPlex Exosome Kit.

2. Protocols for assay performance

▲ Avoid air bubbles.

2.1 Protocol overviews

2.1.1 Short protocol for MACSPlex Filter Plate

Figure 2.1.1: Experimental overview for the short protocol filter plate.

2.1.2 Short protocol for 1.5 mL reagent tubes

Figure 2.1.2: Experimental overview for the short protocol tube.

2.1.3 Overnight protocol for MACSPlex Filter Plate

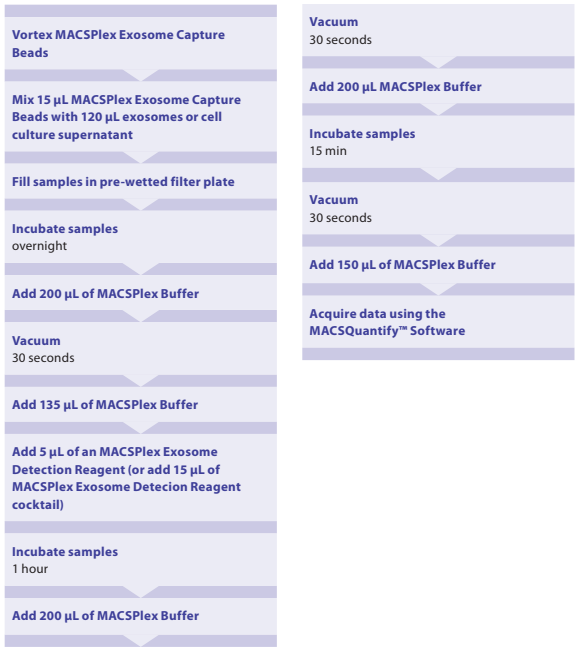

Figure 2.1.3: Experimental overview for the overnight protocol filter plate.

2.1.4 Overnight protocol for 1.5 mL reagent tubes

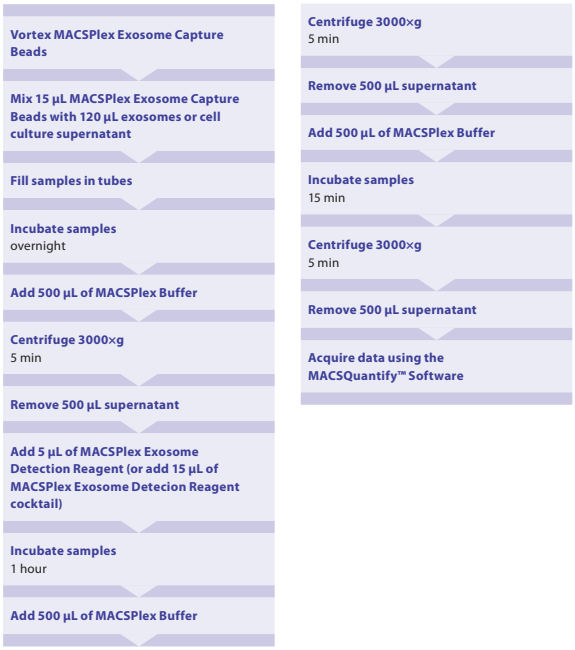

Figure 2.1.4: Experimental overview for the overnight protocol tube.

2.2 Sample preparation

The MACSPlex Exosome Kit can be performed on pre-cleared cell culture supernatant, ascites, or urine directly. For plasma, serum, or cell culture supernatant of cells scarcely secreting exosomes, it is recommended to isolate exosomes beforehand as described below.

▲ Other methods for exosome concentration or isolation like precipitation methods, density gradients, or immunoaffinity purification can also be used.

2.2.1 Pre-clearing cell culture supernatant

1. Incubate the cells of interest in serum-free medium for 12–72 hours depending on the cell line. Adjust the incubation conditions to an apoptosis rate of less than 5%.
2. Remove cells, cell debris, and larger vesicles by serial centrifugations at 300×g for 10 minutes, 2,000×g for 30 minutes, and 10,000×g for 45 minutes.
3. Filter the supernatant through a 0.22 µm membrane.

2.2.2 Exosome isolation using MicroBeads

▲ The isolation does not require ultracentrifugation.

For pre-enrichment of exosomes from plasma we recommend the Exosome Isolation Kit CD63, human (# 130-110-918). For details please refer to the data sheet.

Optionally could be used the Exosome Isolation Kit CD9, human (# 130-110-913) or the Exosome Isolation Kit CD81, human (# 130-110-914).

2.2.3 Exosome isolation from cell culture supernatant (modified according to reference 1)

1. Isolate the exosomes by ultracentrifugation of the pre-cleared supernatant (refer to 2.1.1) at 100,000×g for 2 hours. Resuspend and pool the pellets in a volume of PBS equivalent to supernatant volume and repeat the ultracentrifugation step.
2. Resuspend the exosome pellet in 1/2000 of the original supernatant volume of PBS and determine the exosome concentration indirectly by quantifying the protein concentration.
3. Store the exosomes at –20 °C or –80 °C.

2.2.4 Exosome isolation from plasma (modified according to reference 1)

1. Draw blood into EDTA or citrate tubes (minimum of blood: 10 mL).
2. Separate plasma by centrifugation at 1,000×g for 10 minutes.
3. Dilute plasma with an equal volume of PBS.
4. Remove cells and cellular debris by serial centrifugations at 2,000×g for 30 minutes and 10,000×g for 45 minutes.
5. Isolate the exosomes by ultracentrifugation of the supernatant at 100,000×g for 2 hours. Resuspend and pool the pellets in PBS equal to plasma volume of step 3.
6. Filter the resuspended pellet through a 0.22 µm membrane.
7. Repeat the ultracentrifugation step and resuspend the exosome pellet in 1/250 to 1/500 of the initial volume of PBS. Determine the exosome concentration indirectly by quantifying the protein concentration.
8. Store the exosomes at –20 °C or –80 °C.

2.3 Protocols

▲ MACSPlex Exosome Detection Reagents CD9, CD63, and CD81 could be combined to create a detection cocktail.

2.3.1 Short protocol for the assay using the MACSPlex Filter Plate

▲ Be sure to determine the exosome concentration indirectly by quantifying the protein concentration.

▲ Place the MACSPlex Filter Plate on a non-absorbent surface during loading steps and incubation, i.e., remove any paper towel from the surface to prevent the wells from running dry. Ensure that residual drops under the plate are completely removed to prevent liquid transfer by placing the plate briefly on a paper towel.

▲ Cover unused wells of the filter plate for later use with the adhesive foil provided with the kit.

▲ Use multichannel pipettor.

1. Pre-wet required wells of the MACSPlex Filter Plate with 200 µL of MACSPlex Buffer per well and aspirate off using a vacuum manifold designed to accommodate the filter plate (max. –60 mbar) until the wells are drained or centrifuge 300×g at room temperature for 3 minutes.
2. Place the filter plate briefly on a paper towel to remove residual liquid.
3. Add to each well of the MACSPlex Filter Plate either
   - 120 µL of buffer (blank control) or
   - 120 µL of pre-cleared cell culture supernatant or
   - 120 µL eluate from exosomes isolated with MicroBeads or

- isolated exosomes (4–20 µg protein) from each sample diluted to 120 µL using the MACSPlex Buffer.

4. Resuspend MACSPlex Exosome Capture Beads by vortexing for at least 30 seconds and transfer 15 µL of MACSPlex Exosome Capture Beads to each well.
5. Add 5 µL of MACSPlex Exosome Detection Reagent CD9, CD63, or CD81 or 15 µL of detection cocktail to each well and mix by pipetting up and down.
6. Incubate filter plate for 1 hour at room temperature protected from light on an orbital shaker (450 rpm).
7. Add 200 µL of MACSPlex Buffer to each well.
8. Put the filter plate on a vacuum manifold and apply vacuum (max. –60 mbar) for about 30 seconds until wells are drained. Switch off the vacuum and ventilate the vacuum manifold or centrifuge 300×g at room temperature for 3 minutes.
9. Add 200 µL of MACSPlex Buffer to each well.
10. Incubate filter plate for 15 minutes at room temperature protected from light on an orbital shaker (450 rpm).
11. Put the filter plate on a vacuum manifold and apply vacuum (max. –60 mbar) for about 30 seconds until wells are drained. Switch off the vacuum and ventilate the vacuum manifold or centrifuge 300×g at room temperature for 3 minutes.
12. Add 150 µL of MACSPlex Buffer to each well. Resuspend carefully.

2.3.2 Short protocol for the assay using 1.5 mL tubes

▲ Be sure to determine the exosome concentration indirectly by quantifying the protein concentration.

1. Label reagent tubes for the blank control and samples.
2. Add to each 1.5 mL tube either
   - 120 µL of buffer (blank control) or
   - 120 µL of pre-cleared cell culture supernatant or
   - 120 µL eluate from exosomes isolated with MicroBeads or
   - isolated exosomes (4–20 µg protein) from each sample diluted to 120 µL using the MACSPlex Buffer.
3. Resuspend MACSPlex Exosome Capture Beads by vortexing for at least 30 seconds and transfer 15 µL of MACSPlex Exosome Capture Beads to each tube.
4. Add 5 µL of MACSPlex Exosome Detection Reagent CD9, CD63, or CD81 or 15 µL of detection cocktail to each tube and and mix by pipetting up and down.
5. Incubate tubes for 1 hour at room temperature protected from light using a MACSmix™ Tube Rotator on permanent run (12 rpm) or an orbital shaker (450 rpm).
6. Add 500 µL of MACSPlex Buffer to each tube.
7. Centrifuge at room temperature at 3000×g for 5 minutes.
8. Aspirate 500 µL of the supernatant carefully, leaving about 150 µL in the tube.
9. Add 500 µL of MACSPlex Buffer to each tube.

10. Incubate tubes for 15 minutes at room temperature protected from light using a MACSmix Tube Rotator on permanent run (12 rpm) or an orbital shaker (450 rpm).
11. Centrifuge at room temperature at 3000×g for 5 minutes.
12. Aspirate 500 µL of the supernatant carefully, leaving about 150 µL in the tube.
13. Resuspend sample by pipetting up and down and transfer the samples to a 96-well round bottom plate.

2.3.3 Overnight protocol for the assay using the MACSPlex Filter Plate

▲ Be sure to determine the exosome concentration indirectly by quantifying the protein concentration.

▲ Place the MACSPlex Filter Plate on a non-absorbent surface during loading steps and incubation, i.e., remove any paper towel from the surface to prevent the wells from running dry. Ensure that residual drops under the plate are completely removed to prevent liquid transfer by placing the plate briefly on a paper towel.

▲ Cover unused wells of the filter plate for later use with the adhesive foil provided with the kit.

▲ Use multichannel pipettor.

1. Pre-wet required wells of the MACSPlex Filter Plate with 200 µL of MACSPlex Buffer per well and aspirate off using a vacuum manifold designed to accommodate the filter plate (max. –60 mbar) until the wells are drained or centrifuge 300×g at room temperature for 3 minutes.
2. Place the filter plate briefly on a paper towel to remove residual liquid.

3. Add to each well of the MACSPlex Filter Plate either
   - 120 µL of buffer (blank control) or
   - 120 µL of pre-cleared cell culture supernatant or
   - 120 µL eluate from exosomes isolated with MicroBeads or
   - isolated exosomes (4–20 µg protein) from each sample diluted to 120 µL using the MACSPlex Buffer.
4. Resuspend MACSPlex Exosome Capture Beads by vortexing for at least 30 seconds and transfer 15 µL of MACSPlex Exosome Capture Beads to each well.
5. Incubate filter plate overnight at room temperature protected from light on an orbital shaker (450 rpm).
6. Add 200 µL of MACSPlex Buffer to each well.
7. Put the filter plate on a vacuum manifold and apply vacuum (max. –60 mbar) for about 30 seconds until wells are drained. Switch off the vacuum and ventilate the vacuum manifold or centrifuge 300×g at room temperature for 3 minutes.
8. Add 135 µL of MACSPlex Buffer to each well.
9. Add 5 µL of MACSPlex Exosome Detection Reagent CD9, CD63, or CD81 or 15 µL of detection cocktail to each well and mix by pipetting up and down.
10. Incubate filter plate for 1 hour at room temperature protected from light on an orbital shaker (450 rpm).
11. Add 200 µL of MACSPlex Buffer to each well.
12. Put the filter plate on a vacuum manifold and apply vacuum (max. –60 mbar) for about 30 seconds until wells are drained. Switch off the vacuum and ventilate the vacuum manifold or centrifuge 300×g at room temperature for 3 minutes.

13. Add 200 µL of MACSPlex Buffer to each well.
14. Incubate filter plate for 15 minutes at room temperature protected from light on an orbital shaker (450 rpm).
15. Put the filter plate on a vacuum manifold and apply vacuum (max. –60 mbar) for about 30 seconds until wells are drained. Switch off the vacuum and ventilate the vacuum manifold or centrifuge 300×g at room temperature for 3 minutes.
16. Add 150 µL of MACSPlex Buffer to each well. Resuspend carefully.

2.3.4 Overnight protocol for the assay using 1.5 mL tubes

▲ Be sure to determine the exosome concentration indirectly by quantifying the protein concentration.

1. Label reagent tubes for the blank control and samples.
2. Add to each 1.5 mL tube either
   - 120 µL of buffer (blank control) or
   - 120 µL of pre-cleared cell culture supernatant or
   - 120 µL eluate from exosomes isolated with MicroBeads or
   - isolated exosomes (4–20 µg protein) from each sample diluted to 120 µL using the MACSPlex Buffer.
3. Resuspend MACSPlex Exosome Capture Beads by vortexing for at least 30 seconds and transfer 15 µL of MACSPlex Exosome Capture Beads to each tube.
4. Incubate tubes for overnight at room temperature protected from light using a MACSmix™ Tube Rotator on permanent run (12 rpm) or an orbital shaker (450 rpm).
5. Add 500 µL of MACSPlex Buffer to each tube.

6. Centrifuge at room temperature at 3000×g for 5 minutes.
7. Aspirate 500 µL of the supernatant carefully, leaving about 135 µL in the tube.
8. Add 5 µL of MACSPlex Exosome Detection Reagent CD9, CD63, or CD81 or 15 µL of detection cocktail to each tube and mix by pipetting up and down.
9. Incubate tubes for 1 hour at room temperature protected from light using a MACSmix Tube Rotator on permanent run (12 rpm) or an orbital shaker (450 rpm).
10. Add 500 µL of MACSPlex Buffer to each tube.
11. Centrifuge at room temperature at 3000×g for 5 minutes.
12. Aspirate 500 µL of the supernatant carefully, leaving about 150 µL in the tube.
13. Add 500 µL of MACSPlex Buffer to each tube.
14. Incubate tubes for 15 minutes at room temperature protected from light using a MACSmix Tube Rotator on permanent run (12 rpm) or an orbital shaker (450 rpm).
15. Centrifuge at room temperature at 3000×g for 5 minutes.
16. Aspirate 500 µL of the supernatant carefully, leaving about 150 µL in the tube.
17. Resupend the MACSPlex Exosome Capture Beads by pipetting up and down and transfer the samples to a 96-well round bottom plate.

3. Flow cytometer setup

The kit includes MACSPlex Exosome Setup Beads for setup of flow cytometers.

3.1 Setup of the MACSQuant® Instrument

Calibrate the MACSQuant® Analyzer using MACSQuant Calibration Beads (# 130-093-607). For details, refer to the data sheet of the MACSQuant Calibration Beads.

After successful finishing of the calibration, the MACSQuant Instrument is ready for measurement. No further setup is required. All necessary setup steps are performed automatically during calibration. When running a manual acquisition on the MACSQuant Analyzer or MACSQuant Analyzer 10, we recommend to first use the MACSPlex Exosome Setup Beads to ensure proper recognition of all bead populations.

▲ The kit is not suitable for use with the MACSQuant VYB.

3.2 Setup of other flow cytometers and data acquisition

The analysis of MACSPlex Exosome Kit requires flow cytometers with blue (e.g. 488 nm) and red (e.g. 635 nm) lasers, which are capable of detecting FITC, PE, and APC. For setting up these instruments, the MACSPlex Exosome Setup Beads are included in the kit.

For details refer to the application note “Data acquisition and analysis - General instructions for MACSPlex Exosome Kit” available at www.miltenyibiotec.com/130-108-813.

4. Flow cytometric data analysis

4.1 Calculation of relative qualification of exosome surface markers

The analysis results in a table listing the median signal intensity for all exosome surface markers. The data analysis consists of the following steps:

- Background subtraction
- Calculation of normalization factor

  ▲ **Note:** When isolating with MicroBeads please consider that the respective marker will be blocked, affecting the signal intensity on the MACSPlex Exosome Capture Beads. The signal intensity of that specific marker cannot be used for normalization (steps 3–6).

- Normalization of detected signals
- Determination of relative exosome surface marker levels

1. Subtract the median signal intensity of each bead obtained from your control sample (buffer only) from the signal intensities of the respective beads incubated with your sample.
2. Repeat step 1 for all samples to be analyzed.
3. For each sample calculate the median signal intensity of the signals detected for the MACSPlex Exosome Capture Beads CD9, CD63, and CD81.
4. Use the mean of the median signal intensity of the MACSPlex Exosome Capture Beads CD9, CD63, and CD81 as the normalization factor for each sample.
5. Divide the signal intensities of all beads by the normalization factor of the respective sample.

6. The mean of the MACSPlex Exosome Capture Beads is thereby set to 1 or 100%.
7. Determine the relative exosome marker level by calculating the ratio of the signal intensities of each two samples to be compared.

5. Performance

The assay sensitivity, specificity, and reproducibility of the MACSPlex Exosome Kit was tested on exosomes from cell culture supernatant of primary cells or cancer cell lines as well as on isolated plasma exosomes.

6. Troubleshooting

The following section offers solutions for problems that might be encountered when using the MACSPlex Exosome Kit.

- **Variation between replicate samples:**
  MACSPlex Exosome Capture Beads can settle down. Vortex the MACSPlex Exosome Capture Beads briefly at the latest after pipetting of four samples.
- **Low counts in samples:**
  Mix MACSPlex Exosome Capture Beads sufficiently before pipetting. Ensure that the instrument is calibrated for the relevant 96-well plate to avoid aspiration of air. Avoid aspiration of beads during washing steps. Do not wash or resuspend beads in volumes higher than recommended. Make sure to centrifuge the samples at 3000×g when working with 1.5 mL reagent tubes.
- **Not all expected populations are detected:**
  At least one population could not be detected. Check the liquid level in the wells before starting the measurement. MACSPlex

Exosome Capture Beads can settle down. Vortex the MACSPlex Exosome Capture Beads briefly at the latest after pipetting of your samples. Mix MACSPlex Exosome Capture Beads sufficiently before pipetting. Ensure that the instrument is calibrated for the 96-well filter plate to avoid aspiration of air. Avoid aspiration of beads during washing steps. Do not wash or resuspend beads in volumes higher than recommended. Make sure to centrifuge the samples at 3000×g when working with 1.5 mL reagent tubes. Exosomes comprise several surface epitopes and one exosome can bind to more than one bead. Thereby, two or more beads can be crosslinked via one or more exosomes. High exosome concentrations increase the likelihood of such crosslinking events and the most prominent surface markers will preferentially link the respective beads. Only single beads are used for data acquisition and doublets or aggregates are excluded. For prominent surface markers the number of single beads can drop in case of high exosome concentrations. It is recommended to repeat the experiment with diluted exosome samples (4–20 µg protein diluted in 120 µL of MACSPlex Buffer). Data files have to be analyzed manually. Please refer to the application note "Data acquisition and analysis - General instructions for MACSPlex Exosome Kit" available at www.miltenyibiotec.com/130-108-813.

- **Low counts for some bead populations:**
  Exosomes comprise several surface epitopes and one exosome can bind to more than one bead. Thereby, two or more beads can be crosslinked via one or more exosomes. High exosome concentrations increase the likelihood of such crosslinking events and the most prominent surface markers will preferentially link the respective beads. Only single beads are used for data

acquisition and doublets or aggregates are excluded. For prominent surface markers the number of single beads can drop in case of high exosome concentrations. It is recommended to repeat the experiment with diluted exosome samples (4–20 µg protein diluted in 120 µL of MACSPlex Buffer).

- **High background in buffer control sample:**
  Antibodies can stick non-specifically to the MACSPlex Exosome Capture Beads. Sufficient washing is required to avoid increased background signal intensities. Avoid extended drying of the beads especially if using the filter plate. Immediately add buffer after removing the liquid.
- **High background on isotype control:**
  High concentration of exosomes or contaminations, e.g., from cell culture medium can give rise to non-specific binding of exosomes to the beads. It is recommended to repeat the experiment with diluted exosome samples or to try isolated exosomes instead of cell culture supernatant.
- **Little or no detection of exosomes in sample:**
  When isolating with MiroBeads please consider that the respective marker will be blocked, affecting the signal intensity on the MACSPlex Exosome Capture Beads.
  Signal intensities on the MACSplex Exosome Capture Beads mainly depend on the exosome concentration. Low signal intensities can be indicative for low exosome concentration. Concentrating the exosomes, e.g., by isolation from larger volumes or extended culture times to increase exosome yield could improve signal intensities.
  Prolonged incubation times, e.g., overnight usually enhances

exosome binding and can be used to improve signal intensities. Vigorous vacuuming can strip exosomes from the beads during washing steps using the filter plate on a vacuum station. Lowering the vacuum power could improve the signal to noise ratio. Fluorescent dyes are susceptible to photo bleaching. Avoid prolonged exposure of the fluorescent sample to direct light. Make sure to mix the samples with the reagents during incubation. MACSPlex Exosome Capture Beads tend to sediment and exosomes binding might be insufficient.

- **Beads not in region or gate:**
  Ensure proper calibration of the MACSQuant® Instrument. It is recommended to use the MACSPlex Exosome Setup Beads and to control proper recognition of all bead populations. Instead of an exosome sample, 150 µL of MACSPlex Exosome Setup Beads can be used to control proper bead recognition.
  Samples containing organic solvents or samples of high viscosity should be diluted or dialyzed, respectively.
- **High variation in samples:**
  Pipette may not be calibrated. Washing was not uniform. Samples may have contained high particulate matter or other interfering substances. Plate agitation was insufficient.
  Cross-well contamination could have happened. Change pipette tips for each well when touching the reagent.

- **Filter plate will not vacuum:**
  Vacuum pressure is insufficient. Increase vacuum pressure.
- **Plate leakage:**
  Vacuum pressure is too high. Adjust vacuum pressure to maximal –60 mbar. Place the MACSPlex Filter Plate on a non-absorbent surface during filling steps and incubation, i.e., remove any paper towel from the surface, to prevent the wells from running dry.
  Ensure that residual drops under the plate are completely removed to prevent leakiness of the wells, by placing the plate briefly on a paper towel, after each washing step.
  Avoid touching the plate filter with the tip of the pipette when adding reagents to the wells.

7. Reference

1. Théry, C. *et al.* (2006) Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr. Protoc. Cell Biol.: 3.22.1–3.22.29.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Warranty

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