Miltenyi Biotec

MACSPlex Analysis of plasma-derived extracellular vesicles

Background

Extracellular vesicles (EVs) or exosomes are small membrane vesicles of endocytic origin that are released by many cell types, e.g., T cells, B cells, dendritic cells, platelets, neurons, and epithelial cells[1]. Depending on the originating cell, exosomes are loaded with a specific set of proteins, lipids, and nucleic acids[2]. EVs are involved in various biological processes, including immune surveillance, blood coagulation, neuronal communication, stem cell maintenance, and tissue repair. Their impact on tumor progression, neurodegeneration, autoimmune disorders, and other diseases is under investigation[2]. The surface proteins on EVs can affect the cellular uptake and the EV load can impact the physiology of target cells[3]. Due to their small size, it has been difficult to analyze EVs by standard flow cytometry[1], posing a technical limitation to scientific advancement in this field.
Therefore, we developed the MACSPlex Exosome Kit, human to enable an easy and fast screening of potential EV surface proteins. The combination of 39 different MACSplex Exosome Capture Beads allows for the detection and analysis of 37 markers plus two isotype controls in parallel by standard flow cytometry. While EVs from cell culture supernatant or body fluids like urine or ascites can be directly analyzed, we recommend pre-enriching EVs from human plasma using the Exosome Isolation Kit CD9, human, Exosome Isolation Kit CD63, human, or Exosome Isolation Kit CD81, human as described in this application note.

Principle of the Exosome Isolation Kits, human

The isolation of exosomes or EVs is performed by positive selection using MicroBeads recognizing the tetraspanin proteins CD9, CD63, or CD81. First, EVs are magnetically labeled with Exosome Isolation MicroBeads CD9, CD63, or CD81 during a short incubation period. The labeled EVs are loaded onto a µ Column, which is placed in the magnetic field of a µMACS® Separator. The magnetically labeled EVs are retained within the column, while the unlabeled vesicles and cell components run through. After removing the column from the magnetic field, the intact EVs can be eluted (fig. 1).

Principle of the MACSplex Exosome Kit, human

The MACSPlex Exosome Kit comprises a cocktail of various fluorescently labeled bead populations, the MACSplex Capture Beads, which can be distinguished by flow cytometry. Each of these MACSplex Capture Bead populations is coupled to a specific antibody binding to a respective exosomal surface epitope. Exosomes bound to the MACSplex Capture Beads are stained with APC-conjugated antibodies, e.g., a cocktail of antibodies against the tetraspanins CD9, CD63, and CD81. This leads to the formation of complexes, each consisting of i) MACSplex Capture Bead, ii) exosome, and iii) APC-conjugated antibody (fig. 2). These complexes can then be analyzed based on the fluorescence characteristics of both the MACSPlex Capture Beads and the APC-conjugated antibodies.
As there are 39 MACSplex Capture Bead populations, 39 signal values are measured for each single sample. Positive APC signals detected in a given sample thus indicate that the epitopes captured by the antibodies coupled to the MACSplex Capture Beads in that sample are present on the exosome surface.

Materials and methods

Reagents and solutions

- Exosome Isolation Kit CD63, human (# 130-110-918) or Exosome Isolation Kit CD81, human (# 130-110-914) or Exosome Isolation Kit CD9, human (# 130-110-913)
- MACSplex Exosome Kit (# 130-108-813)

Materials

- MACSQuant® Analyzer, 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.

- Chill 96 Rack (# 130-094-459), when using the MACSQuant Analyzer or MACSQuant Analyzer 10.
- MACSQuant Calibration Beads (# 130-093-607), when using the MACSQuant Analyzer or MACSQuant Analyzer 10.

- MACSmix™ Tube Rotator (# 130-090-753)
- µMACS™ Separator (# 130-042-602)
- MACS® MultiStand (# 130-042-303)
- Centrifuge
- 1.5 mL tubes
- Optional: Membrane filter (0.22 µm pore size)

Procedure

Isolation of extracellular vesicles from plasma

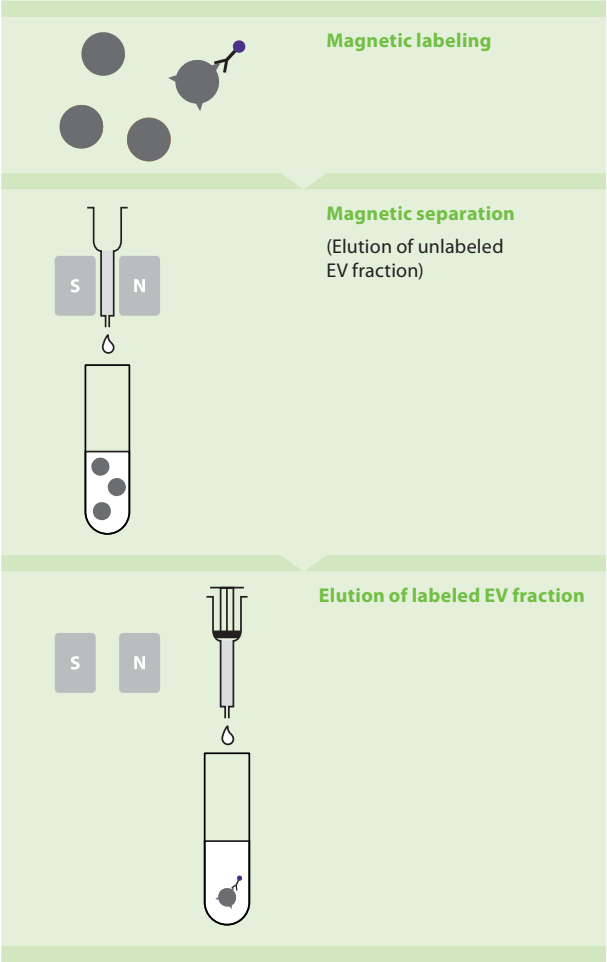

Figure 1: Principle of magnetic isolation of EVs using an Exosome Isolation Kit.

1. Centrifugation of whole blood to obtain pre-cleared plasma.
2. 1 h incubation with Exosome Isolation MicroBeads CD9, human, Exosome Isolation MicroBeads CD63, human, or Exosome Isolation MicroBeads CD81, human.
3. Magnetically labeled sample is run through a µ Column placed in the magnetic field of the µMACS Separator.
4. Unbound plasma components are washed off the column.
5. Elution of EVs by removing the column from the magnetic separator and adding Exosome Isolation Buffer onto the column.

For a more detailed protocol, please refer to the corresponding data sheet at **www.miltenyibiotec.com**.

MACSplex Exosome Assay

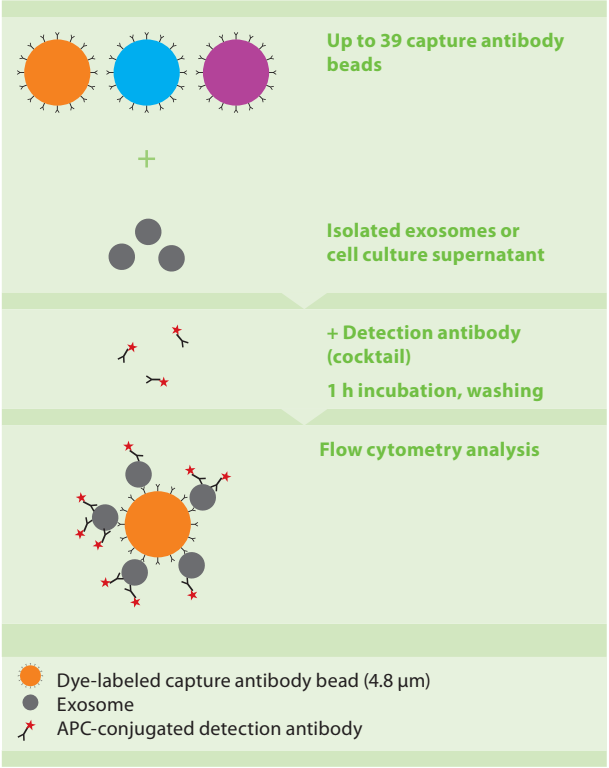

Figure 2: Principle of the MACSPlex Exosome Kit.

1. Isolated EVs (eluate from plasma isolation) are incubated for 1 h with MACSPlex Capture Beads specific for 37 different exosomal surface markers (plus two isotype control beads) and the MACSPlex Exosome Detection Reagent CD9, CD63 and CD81 cocktail comprising the three APC-conjugated exosome detection antibodies.
2. After removing unbound exosomes and detection reagent, the samples can be used for flow cytometric analysis.

For detailed protocols see the MACSPlex manual.

Note: Following the MACSPlex protocol, samples can be additionally fixed before the flow cytometric measurement. This can be done in a filter plate or a tube. Detailed protocols can be found at **www.miltenyibiotec.com**.

Flow cytometric acquisition using the MACSQuant® Express Modes

To perform the acquisition and data analysis of the MACSPlex Exosome Assay with the MACSQuant® Analyzers use the Express Mode "MACSPlex_Exosome" to achieve automated measurement and generation of primary data. For details refer to the special protocol "Data acquisition and analysis of MACSPlex Exosome Kit using the MACSQuant Analyzer Express Mode" available at **www.miltenyibiotec.com/130-108-813**.

Results

EVs from 2 mL of plasma were isolated using CD9, CD63, or CD81 MicroBeads and analyzed by the MACSPlex Exosome Kit (fig. 3). For comparison, EVs were isolated by ultracentrifugation. Amounts were adjusted to a plasma volume of 2 mL and subsequently analyzed using the same assay.

For most of the MACSPlex Exosome Capture Bead types, EVs isolated by CD63 MicroBeads gave the strongest signals, followed by CD81 and CD9 MicroBeads.

Note: After exosome isolation using MicroBeads, the respective epitope is predominantly blocked by MicroBeads and low signals will be detected on the respective MACSPlex Bead Type (e.g. CD9, CD63, or CD81). Therefore, the Exosome Isolation Kit Pan, human, which contains a cocktail of CD9, CD63, and CD81 MicroBeads, is not recommended to be used prior to the MACSPlex Exosome Kit, human. Instead we recommend using one of the single tetraspanin Exosome Isolation Kits (Exosome Isolation Kit CD9, human, Exosome Isolation Kit CD63, human, or Exosome Isolation Kit CD81, human).

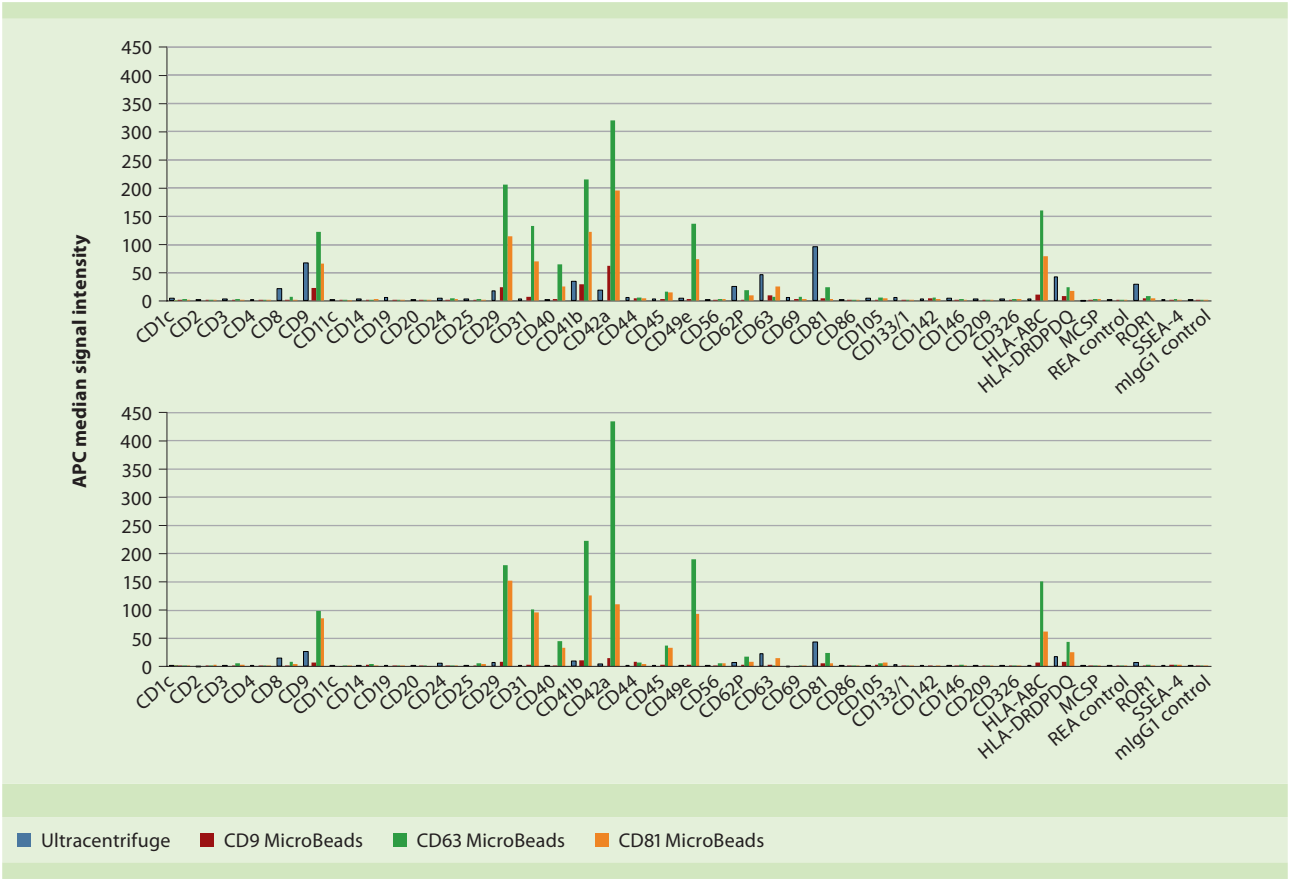

Figure 3: Surface marker profile of EVs isolated from plasma of donor A (top) or B (bottom) by ultracentrifugation or immunomagnetic isolation using CD9, CD63, or CD81 MicroBeads. Data indicate APC median signal intensities of isolated EVs incubated with the 39 MACSplex Exosome Capture Beads and stained with a cocktail of CD9-, CD63-, and CD81-APC antibodies. REA and mIgG1 indicate isotype control MicroBeads.

Conclusion

- Superparamagnetic MicroBeads enable the isolation of EVs from plasma.
- Magnetically isolated EVs from human plasma can be analyzed by the MACSPlex Assay.
- The MACSPlex Assay allows for the specific detection of EV surface proteins on EVs isolated from plasma.

References

1. Raposo, G. and Stoorvogel, W. (2013) Extracellular vesicles: exosomes, microvesicles, and friends. J. Cell Biol., 200: 373–383.
2. Andaloussi, S.E.L. *et al.* (2013) Extracellular vesicles: biology and emerging therapeutic opportunities. Nat. Rev. Drug Discov. 12: 347–357.
3. Thery, C. *et al.* (2009) Membrane vesicles as conveyors of immune responses. Nat. Rev. Immunol. 9: 581–593.

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