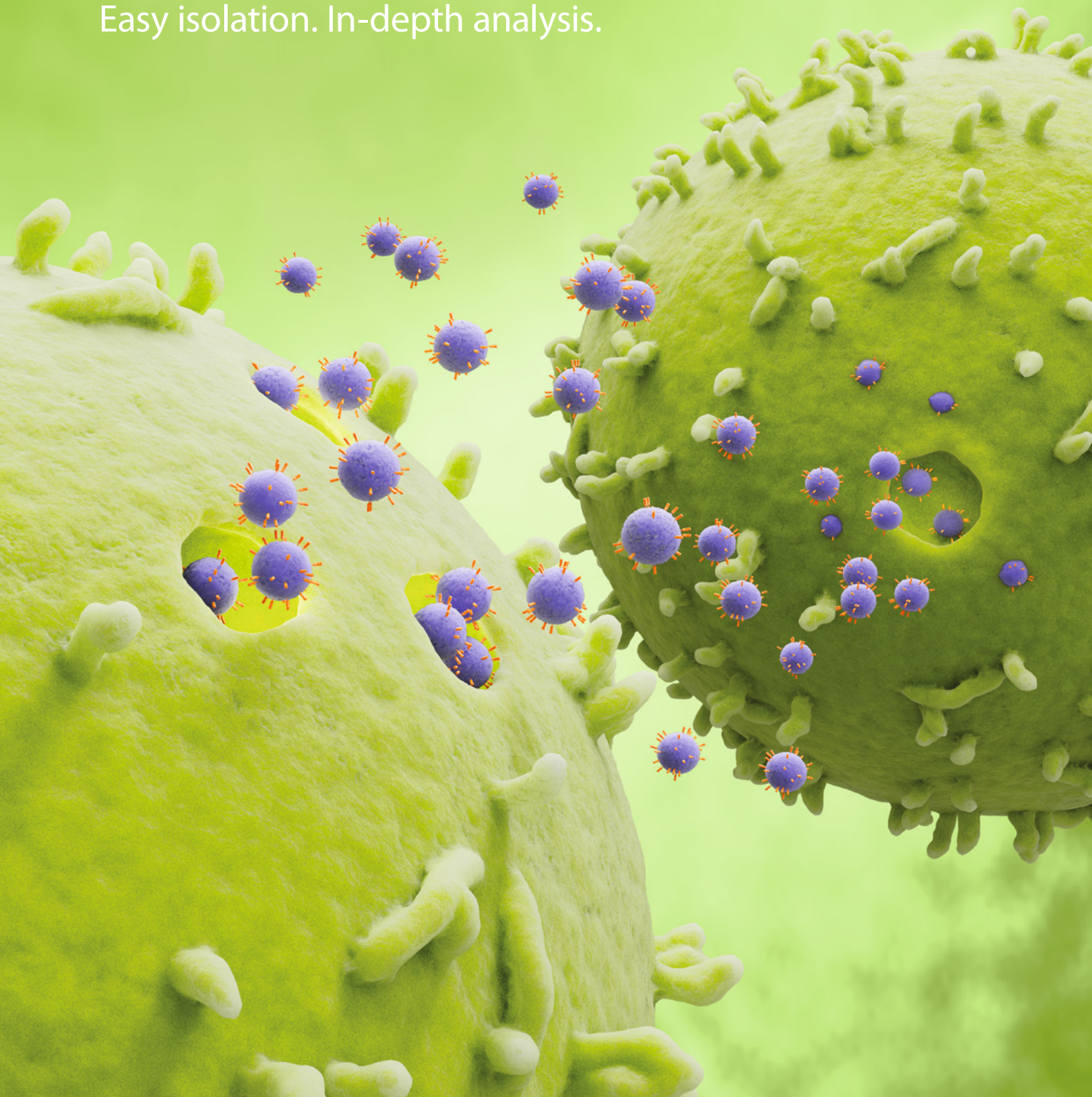
Miltenyi Biotec

Extracellular vesicle solutions

Easy isolation. In-depth analysis.



Isolating extracellular vesicles the easy way

Without ultracentrifugation

Isolation of extracellular vesicles from cell culture supernatant or body fluids

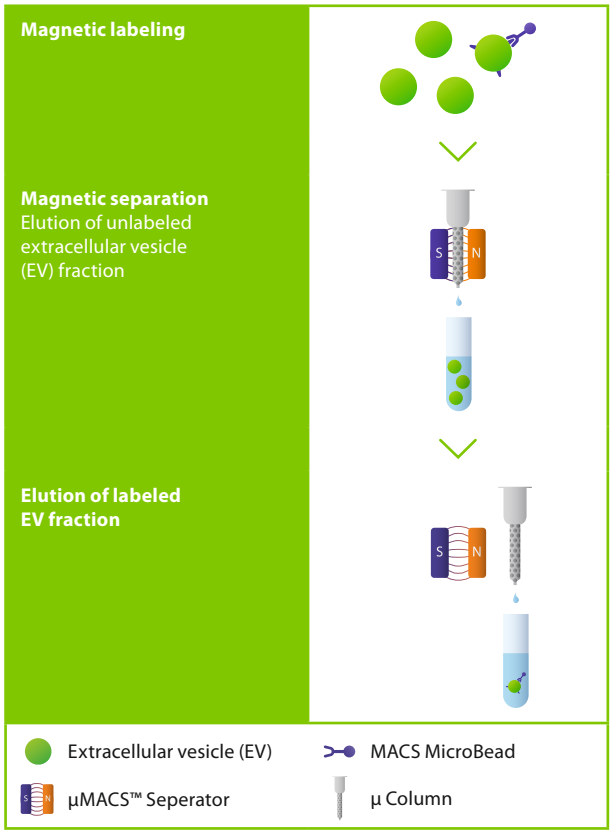


Figure 1: Principle of magnetic isolation of EVs using an EV Isolation Kit.

Extracellular vesicles – Start small. Discover big.

Extracellular vesicles (EVs), including exosomes, are secreted by virtually every cell type and can be found in all body fluids. They carry various molecules and play an important role as intercellular messengers in health and disease.
There is growing interest in applying EVs in clinical settings as they can potentially be used as diagnostic biomarkers and therapeutic agents.

MACS® Technology – the proven technique for magnetic separation

EVs can be easily isolated by MACS Technology – the tried and tested cell isolation method cited in tens of thousands of publications. This technique owes its longstanding success to the combination of three components: i) nano-sized superparamagnetic MACS MicroBeads coupled to antibodies specifically detecting certain epitopes on the surface of cells or EVs, ii) MACS Separators, and iii) MACS Columns generating a strong magnetic field.

Miltenyi Biotec offers dedicated kits for isolating EVs based on the tetraspanin proteins CD9, CD63, and CD81, which are known to be present on the surface of EVs. EV Starting Kits contain all materials required for convenient EV isolation, including separator and columns.

Isolation of EVs in three easy steps

First, EVs contained in cell culture supernatant or body fluids are magnetically labeled with EV Isolation MicroBeads CD9, CD63, or CD81. The labeled EVs are then loaded onto a µ Column, which is placed in the magnetic field of a µMACS™ Separator. Magnetically labeled EVs are retained within the column, while unlabeled vesicles and cell components run through. After removing the column from the magnetic field, the intact EVs can be eluted (fig. 1).

- Fast (< 2h) and easy isolation of EVs without ultracentrifugation
- Targeted isolation based on CD9, CD63, or CD81, or all three makers combined
- EV isolation from cell culture supernatant or body fluids like plasma, urine, or ascites

New horizons for EV analysis

Fast screening by flow cytometry

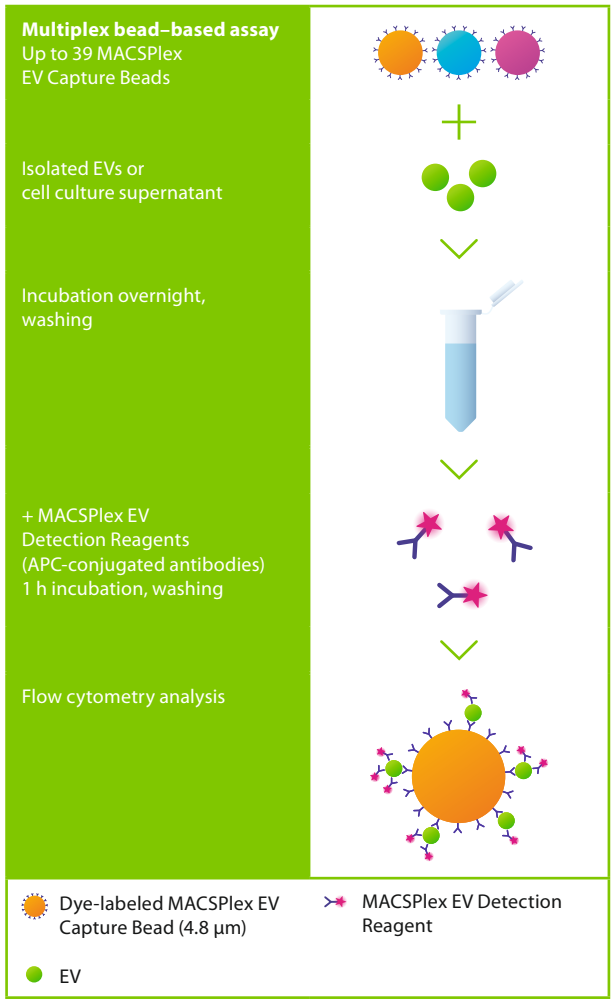


Figure 2: Principle of the MACSPlex EV Kits. Isolated EVs are incubated overnight with 39 differently labeled MACSPlex EV Capture Beads each coupled to a different antibody. EVs bound to the beads are detected with MACSPlex EV Detection Reagents by flow cytometry.

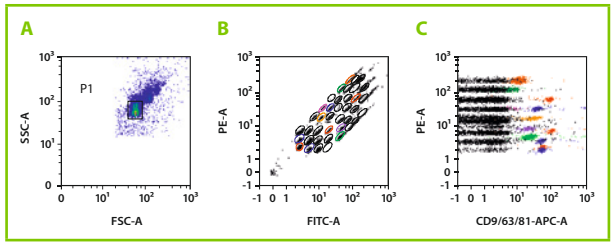


Figure 3: EV analysis based on the MACSPlex EV Kit.
The example shows (A) gating according to bead size,
(B) discrimination of differently labeled bead populations, and
(C) measurement of signal intensities of the single bead populations.

MACSPlex EV Kits – the solution for comprehensive analysis

Due to their small size, it has been difficult thus far to analyze EVs by standard flow cytometry, which hampered scientific advancement in this field.
To facilitate comprehensive EV analysis, Miltenyi Biotec developed the MACSPlex EV Kit. This well-established tool enables an easy and fast screening of potential EV surface proteins across multiple research applications.

- Unique multiplex bead platform for protein profiling of EVs from different origins by flow cytometry
- Saves precious sample material by screening 37 surface markers simultaneously
- Analysis of EVs from cell culture supernatant or body fluids
- Available for immune-oncology (IO), neurobiology (Neuro), and mesenchymal stem cell (MSC) applications.

Principle of the MACSPlex EV Kits

The MACSPlex EV Kit allows for the simultaneous detection of 37 surface epitopes that are known to be present on different EVs. The kit is based on a cocktail of various fluorescently labeled bead populations, the MACSPlex EV Capture Beads, which can be distinguished by flow cytometry.

Each of these MACSPlex EV Capture Bead populations is coupled to a specific antibody binding to a respective EV surface epitope. EVs bound to the MACSPlex Capture Beads are stained with MACSPlex EV Detection Reagents, i.e., a cocktail of APC-conjugated antibodies against the tetraspanins CD9, CD63, and CD81. This leads to the formation of complexes, each consisting of i) MACSPlex EV Capture Bead, ii) EV, and iii) APC-conjugated antibodies (fig. 2). These complexes can then be analyzed based on the fluorescence characteristics of both the MACSPlex EV Capture Beads and the APC-conjugated antibodies (fig. 3). Two isotype control beads are included in the kit to check for non-specific binding.

EV Isolation Kits

For efficient downstream analysis



Fast and reliable EV isolation and protein profiling

EVs isolated with EV Isolation Kits based on single tetraspanins, i. e., CD9, CD63, or CD81, can be easily analyzed for their protein profiles using the MACSPlex EV Kit (fig. 4). For most of the MACSPlex EV Capture Bead types, EVs isolated based on CD63 gave the strongest signals, followed by CD81 and CD9. EVs are traditionally prepared by ultracentrifugation. However, this method is time consuming and can lead to inconclusive results in protein profiling experiments (fig. 4): EVs isolated by ultracentrifugation showed only weak fluorescence signals compared to EVs isolated by MACS Technology (table 1), when analyzed with the MACSPlex EV Kit IO (table 3).

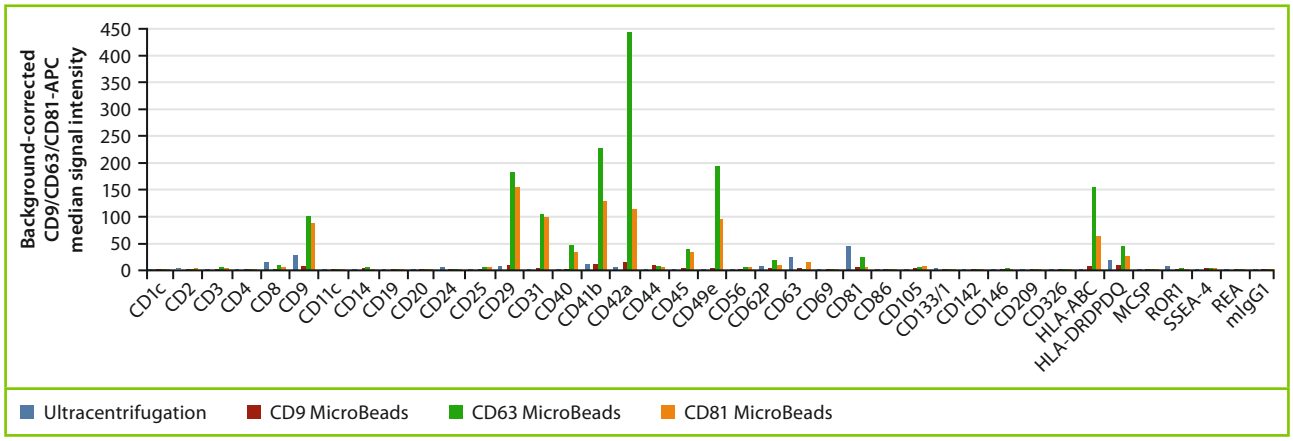


Figure 4: Surface marker profiles of EVs isolated from plasma by ultracentrifugation or immunomagnetic isolation using the EV Isolation Kits CD9, CD63, or CD81. EVs from 2 mL of plasma were isolated using one of the kits or by ultracentrifugation. Amounts were adjusted to a plasma volume of 2 mL, and EVs were analyzed using the MACSPlex EV Kit IO. Data indicate median APC signal intensities of isolated EVs incubated with the 39 MACSPlex EV Capture Beads and stained with a cocktail of CD9-, CD63-, and CD81-APC antibodies. REA and mIgG1 indicate isotype control beads.

Product	Order no.
EV Isolation Kit CD9, human	130-110-913
EV Isolation Kit CD63, human	130-110-918
EV Isolation Kit CD81, human	130-110-914
EV Isolation Kit Pan, human	130-110-912

Table 1: Products for isolating EVs.

Product	Order no.
EV Isolation Kit CD9, mouse	130-117-042
EV Isolation Kit CD63, mouse	130-117-041
EV Isolation Kit CD81, mouse	130-117-040
EV Isolation Kit Pan, mouse	130-117-039

EV Starting kits including µMACS Separator and MultiStand, are also available for all three markers.

MACSPlex EV Kits

For comprehensive EV profiling

Antibodies for EV analysis with MACSPlex EV Kit IO, human		
HLA-ABC	CD19	CD62P
HLA-DR, DP, DQ	CD20	CD63
MCSP	CD24	CD69
ROR1	CD25	CD81
SSEA-4	CD29	CD86
CD1c	CD31	CD105
CD2	CD40	CD133/1
CD3	CD41b	CD142
CD4	CD42a	CD146
CD8	CD44	CD209
CD9	CD45	CD326
CD11c	CD49e	Mouse IgG1 Control
CD14	CD56	REA Control

Table 2: Overview of surface marker and control antibodies used for EV analysis by the MACSPlex EV Kit IO, human.

Product	Order no.
MACSPlex EV Kit IO, human	130-108-813
MACSPlex EV Kit IO, mouse	130-122-213
MACSPlex EV Kit Neuro, human	130-133-425
MACSPlex EV Kit MSC, human	130-136-864

Table 3: Products for characterizing surface markers on EVs by flow cytometry.

EVs from plasma samples

The surface signature of EVs has been the focus of many studies to better understand the heterogenous nature of EVs, identify potential EV subsets with therapeutic activity and reveal disease-specific biomarkers. However, protein profiling of EVs requires a reliable and robust method for the analysis of multiple markers.

The MACSPlex EV Kits are the ideal solution enabling the screening of up to 37 markers in parallel by flow cytometry from cell culture supernatant or body fluid-derived EVs.

Figure 5 shows a protein profile of plasma-derived EVs from one donor at different input doses. Many markers that are expressed on specific blood cell types, like CD20 (B cells) or CD8 (T cells), are also present on plasma-derived EVs. As expected, signal intensities for all positively stained bead populations decreases with decreasing input. Therefore, the expression of EV surface markers can be detected in a robust, sensitive, and reproducible way. Table 2 provides an overview of the antibodies used for EV analysis.

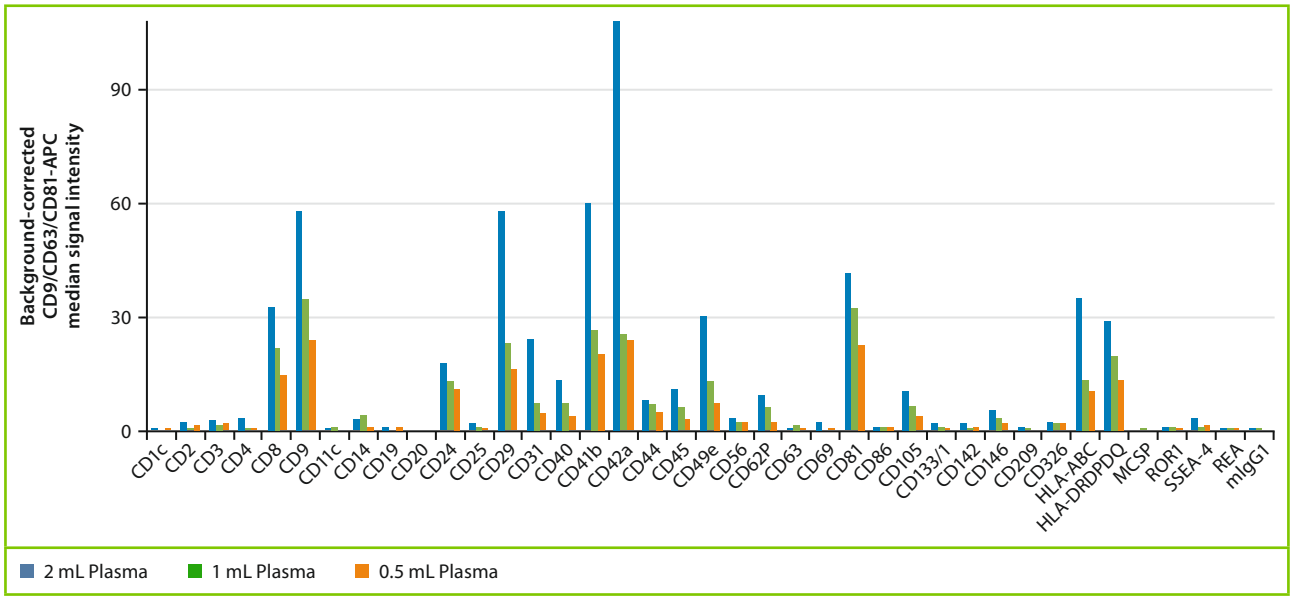


Figure 5: Characterization of plasma-derived EVs with the MACSPlex EV Kit IO, human. Plasma EVs from one donor were isolated with the EV Isolation Kit CD63, human. Different volumes of plasma were used. Isolated plasma EVs were analyzed with the MACSPlex EV Kit IO, human. Data indicate median APC signal intensities of plasma EVs incubated with the 39 MACSPlex EV Capture Beads and stained with a cocktail of CD9-, CD63-, and CD81-APC antibodies. REA and mIgG1 indicate isotype control beads.

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