Exosome research solutions
Magnetic isolation and fast screening
**Isolating exosomes the easy way**

*Without ultracentrifugation*

**Isolation of extracellular vesicles from cell culture supernatant or body fluids**

- **Magnetic labeling**
- **Magnetic separation**
  - Elution of unlabeled EV fraction
- **Elution of labeled EV fraction**

![Diagram](https://example.com/diagram.png)

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

**Exosomes – small vesicles, big impact**

Exosomes are extracellular vesicles (EVs) of endocytic origin, released by numerous cell types including immune cells, stem cells, and neurons. They carry various molecules and play an important role as intercellular messengers in health and disease. There is growing interest in applying exosomes 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 exosomes. Exosome Starting Kits contain all materials required for convenient exosome 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 Exosome 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 exosomes 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 exosome analysis

Fast screening by flow cytometry

MACS®Plex Exosome Kit – 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 MACS®Plex Exosome Kit. This novel tool enables an easy and fast screening of potential EV surface proteins.

- Unique multiplex bead platform for protein profiling of EVs by flow cytometry
- Saves precious sample material by screening 37 surface markers simultaneously
- Analysis of EVs from cell culture supernatant or body fluids

Principle of the MACS®Plex Exosome Kit

The MACS®Plex Exosome 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 MACS®Plex Exosome Capture Beads, which can be distinguished by flow cytometry.

Each of these MACS®Plex Exosome Capture Bead populations is coupled to a specific antibody binding to a respective exosomal surface epitope. Exosomes bound to the MACS®Plex Capture Beads are stained with MACS®Plex Exosome 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) MACS®Plex Exosome Capture Bead, ii) exosome, and iii) APC-conjugated antibodies (fig. 2). These complexes can then be analyzed based on the fluorescence characteristics of both the MACS®Plex Exosome Capture Beads and the APC-conjugated antibodies (fig. 3). Two isotype control beads are included in the kit to check for non-specific binding.
Exosome Isolation Kits
For efficient downstream analysis

Fast and reliable EV isolation and protein profiling
EVs isolated with Exosome Isolation Kits based on single tetraspanins, i.e., CD9, CD63, or CD81, can be easily analyzed for their protein profiles using the MACSplex Exosome Kit (fig. 4). For most of the MACSplex Exosome 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 Exosome Kit (table 3).

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

Table 1: Products for isolating EVs.

Exosome Starting kits including µMACS Separator and MultiStand, are also available for all three markers.
**MACSPlex Exosome Kit**
For comprehensive EV profiling

### Antibodies for EV analysis

<table>
<thead>
<tr>
<th>Antibodies for EV analysis</th>
<th>CD19</th>
<th>CD62P</th>
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</thead>
<tbody>
<tr>
<td>HLA-ABC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DR, DP, DQ</td>
<td>CD20</td>
<td>CD63</td>
</tr>
<tr>
<td>MCSP</td>
<td>CD24</td>
<td>CD69</td>
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<td>ROR1</td>
<td>CD25</td>
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<td>SSEA-4</td>
<td>CD29</td>
<td>CD86</td>
</tr>
<tr>
<td>CD1c</td>
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<td>CD105</td>
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<td>CD2</td>
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<td>CD3</td>
<td>CD41b</td>
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<td>CD9</td>
<td>CD45</td>
<td>CD326</td>
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<tr>
<td>CD11c</td>
<td>CD49e</td>
<td>Mouse IgG1 Control</td>
</tr>
<tr>
<td>CD14</td>
<td>CD56</td>
<td>REA Control</td>
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</table>

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

### EVs from plasma samples

The surface signature of EVs has been the focus of many studies to better understand the heterogeneous 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 Exosome 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 CD24 (B cells) or CD8 (T cells), are also present on plasma-derived EVs. As expected, signal intensities for all positively stained bead populations decrease 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.

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

<table>
<thead>
<tr>
<th>Product</th>
<th>Order no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MACSPlex Exosome Kit, human</td>
<td>130-108-813</td>
</tr>
<tr>
<td>MACSPlex Exosome Kit, mouse</td>
<td>130-122-213</td>
</tr>
</tbody>
</table>

**Figure 5: Characterization of plasma-derived EVs with the MACSPlex Exosome Kit, human.** Plasma EVs from one donor were isolated with the Exosome Isolation Kit CD63, human. Different volumes of plasma were used. Isolated plasma EVs were analyzed with the MACSPlex Exosome Kit, human. Data indicate median APC signal intensities of plasma EVs incubated with the 39 MACSPlex Exosome Capture Beads and stained with a cocktail of CD9-, CD63-, and CD81-APC antibodies. REA and mlgG1 indicate isotype control beads.