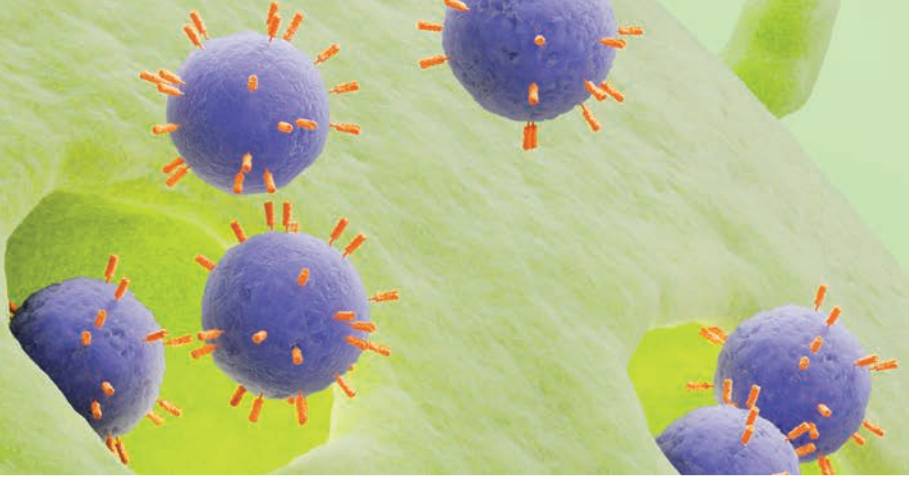




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# The combination of Exosome Isolation Kits and the MACSPlex Exosome Kit allows the phenotyping of EVs released during physical exercise

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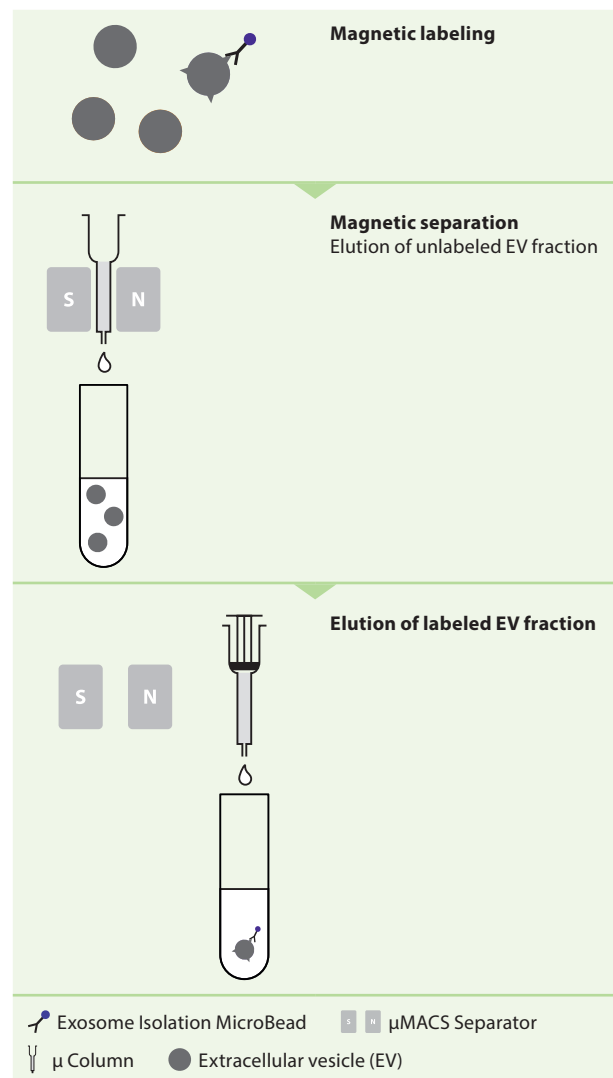
## Background

Extracellular vesicles (EVs) play a key role in cell-cell communication. During physical exercise, EVs are released into the circulation. These so-called Extracellular Vesicles Released during Exercise (ExerVs) may mediate signaling processes associated with the exercise adaptive response and could therefore be valuable biomarkers or therapeutic vehicles for sedentary lifestyle-associated diseases. Here, we utilized Miltenyi Biotec's Exosome Isolation Kit combined with the MACSPlex Exosome Kit to phenotype different ExerV subtypes<sup>1</sup>.

## Methods

### Exosome Isolation Kits

Miltenyi Biotec's Exosome Isolation Kits enable the positive immunomagnetic enrichment of EVs by 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  $\mu$  Column, which is placed in the magnetic field of a  $\mu$ MACS™ 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).



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

### MACSPlex Exosome Kit, human

The MACSPlex Exosome Kit, human is a multiplex bead-based flow cytometry assay, which allows the detection of 37 surface proteins on EVs. The assay contains a cocktail of different fluorescently labeled bead populations, named MACSPlex Exosome Capture Beads, which can be distinguished by flow cytometry. Each of these MACSPlex Exosome Capture Bead populations is coupled to an antibody specifically targeting one of the 37 surface epitopes. EVs that bind to the MACSPlex Exosome Capture Beads are then stained with a detection reagent, e.g., a cocktail of APC-conjugated antibodies against the tetraspanins CD9, CD63, and CD81, which are commonly observed on exosomes. By virtue of the fluorescence properties of both the MACSPlex Exosome Capture Beads and the APC-conjugated antibodies, the flow cytometric semi-quantitative analysis of the proteins expressed on the EVs is then possible (fig. 2).

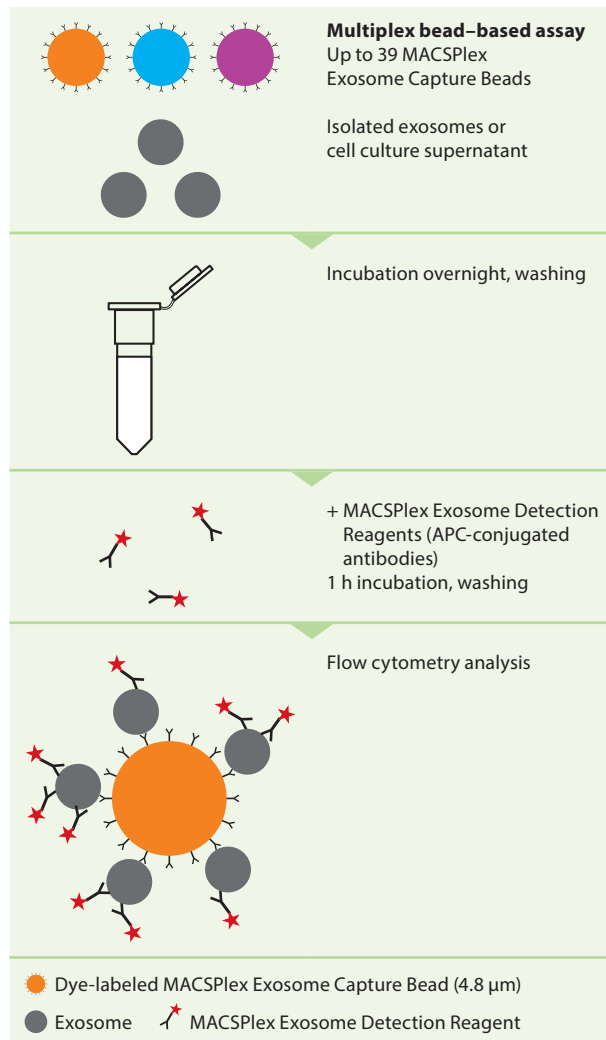


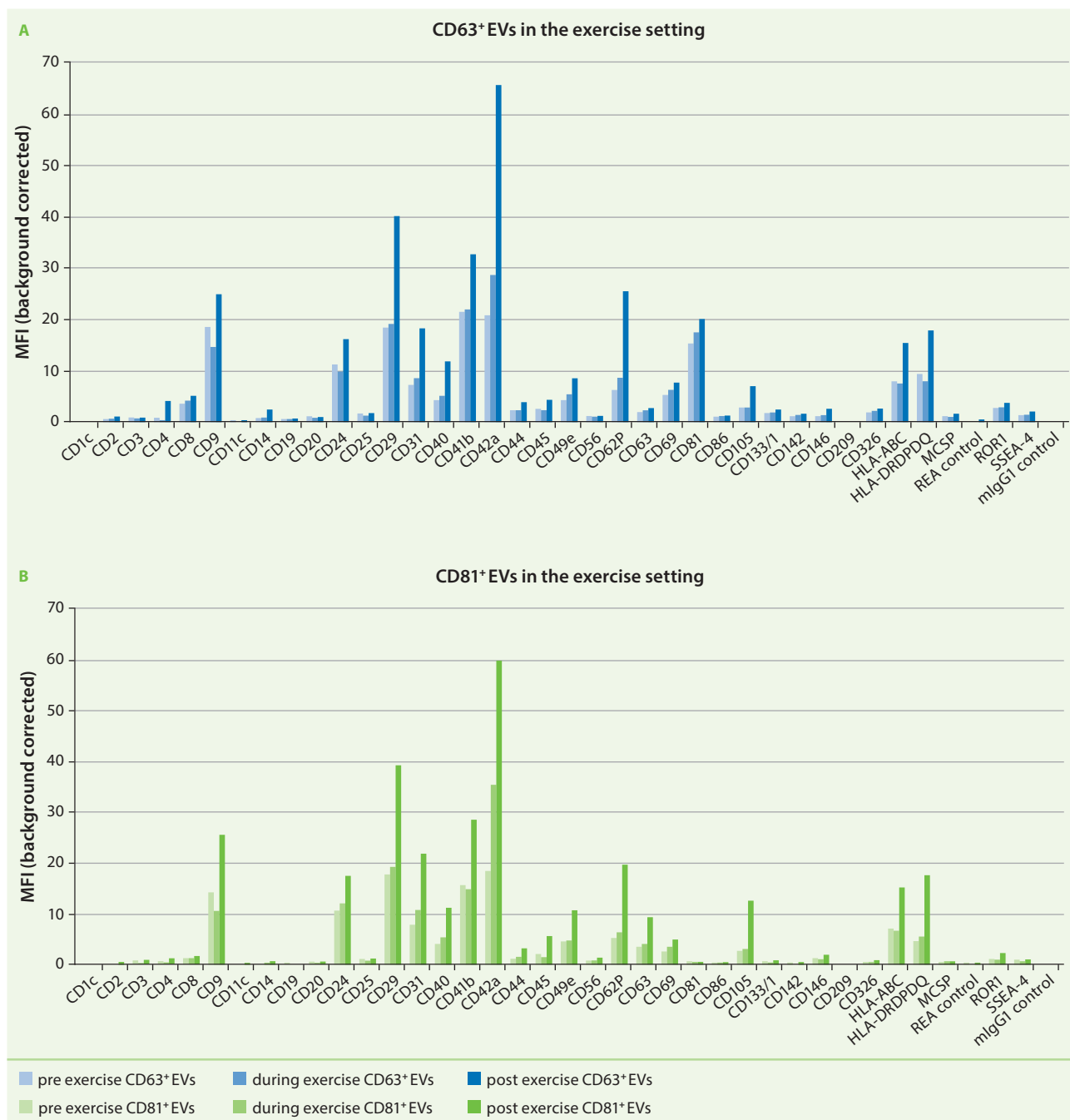
Figure 2: Principle of the MACSPlex Exosome Kit, human

## Results

### Isolation of CD9<sup>+</sup>, CD63<sup>+</sup>, and CD81<sup>+</sup> ExerV subpopulations with Exosome Isolation Kits

To profile EVs released during physical activity, we investigated platelet-free plasma samples of 21 active healthy male subjects. After one night of fasting, samples were collected before, during, and after an incremental cycling test until exhaustion.

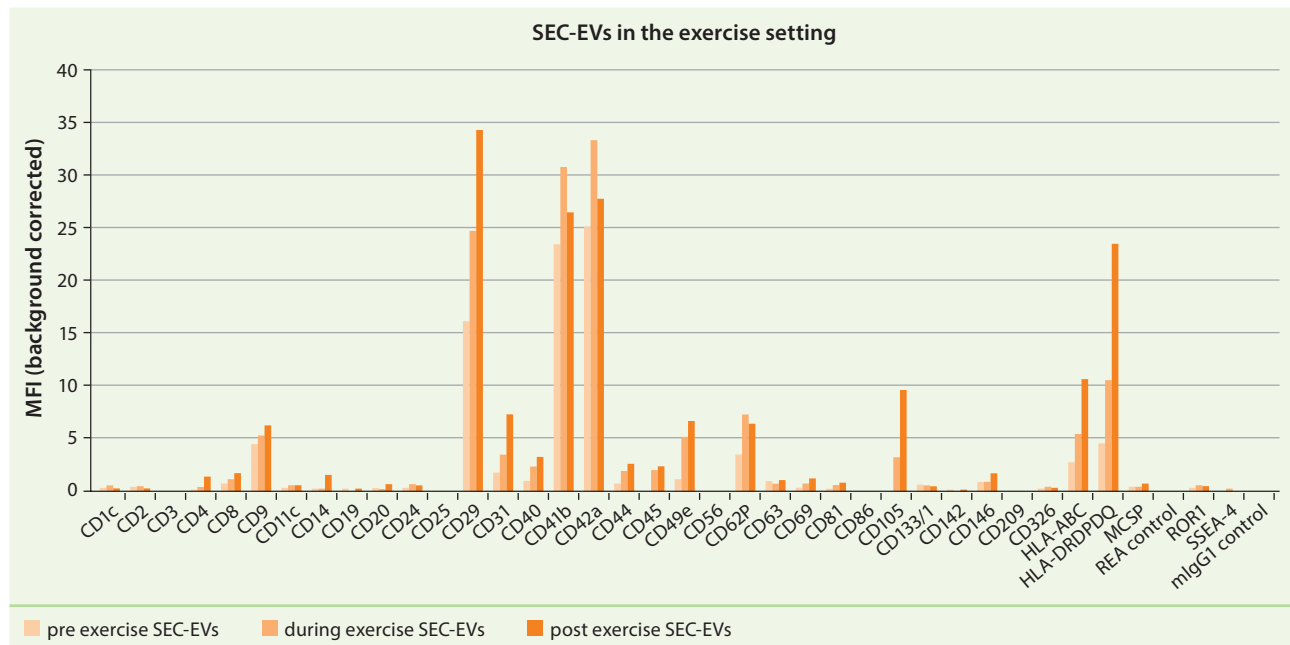
Defined populations of CD9<sup>+</sup> EVs (results not shown), CD63<sup>+</sup> EVs, and CD81<sup>+</sup> EVs could be detected (fig. 3). In general, marker profiles of CD63<sup>+</sup> EVs and CD81<sup>+</sup> EVs show a very similar pattern with respect to high signal intensities for platelet (e.g. CD42a), endothelial (e.g. CD105), and leukocyte markers (e.g. CD40), suggesting the related origin of ExerVs. Notably, markers were generally higher post exercise, indicating the release of EVs into the circulation due to physical activity. Taken together, these findings reveal that the use of Exosome Isolation Kits results in efficient enrichment of CD9<sup>+</sup>, CD63<sup>+</sup>, and CD81<sup>+</sup> ExerVs from plasma samples. Furthermore, analysis with the MACSPlex Exosome Kit, human allowed the investigation of the ExerV phenotypes, which indicated that ExerVs originate, for example, from leukocytes, endothelial cells, and platelets.



**Figure 3: Phenotyping of CD63<sup>+</sup> and CD81<sup>+</sup> ExerVs isolated with respective Exosome Isolation Kits:** Different EV subtypes were isolated with the respective Exosome Isolation Kit before, during, and after physical exercise. EV protein profiles were then analyzed with the MACSPlex Exosome Kit-human. Representative mean fluorescence intensities (MFI) are shown.

## ExerVs isolated with Exosome Isolation Kits show stronger marker signals than EVs isolated via SEC

ExerV protein profiles obtained after isolation with Exosome Isolation Kits (fig. 3) versus size exclusion chromatography (SEC) (fig. 4) show stronger marker signals with robust signal intensities.



**Figure 4: Phenotyping of ExerVs isolated by SEC:** EVs were isolated by SEC before, during, and after physical exercise. EV protein profiles were then analyzed with the MACSPlex Exosome Kit: human. Representative mean fluorescence intensities 'MFI' are shown.

## Conclusions

- Exosome Isolation Kits in combination with the MACSPlex Exosome Kit allow reliable ExerV isolation from plasma samples and subsequent protein profiling.
- ExerV protein profiles of EVs isolated with the Exosome Isolation Kits were associated to stronger signals than ExerVs isolated by SEC.
- ExerV analysis with the MACSPlex Exosome Kit could be beneficial to investigate exercise-triggered processes including cardiovascular function, immune modulation, inflammation-associated tissue regeneration, and regulation of coagulation.

## Reference

- Brahmer A. *et al.* (2019) Platelets, endothelial cells and leukocytes contribute to the exercise-triggered release of extracellular vesicles into the circulation. *J. Extracell. Vesicles* 8:1. DOI: <https://www.tandfonline.com/doi/full/10.1080/20013078.2019.1615820>



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