



Miltenyi Biotec

## Reference list

# Magnetic isolation and fast screening of extracellular vesicles by flow cytometry

Exosomes are extracellular vesicles (EVs) of endocytic origin and released by numerous cell types, including immune cells, stem cells, and neurons. They carry various molecules capable of cellular signaling and are involved in many normal and pathological processes. Moreover, they have received considerable attention due to their potential use in diagnosis and therapy of cancer, infectious diseases, and neurodegenerative disorders. However, isolation and analysis of EVs is made challenging by their small size and heterogeneous nature. Thus, Miltenyi Biotec's Exosome Isolation Kits are indispensable for pure and efficient EV isolation and high-quality downstream analysis, including protein analysis, electron microscopy, and RNA sequencing. The MACSPlex Exosome Kit further allows detection and semi-quantification of 37 surface markers on EVs simultaneously by flow cytometry.

## Selected references

### Pure and fast isolation of EVs

Dagur, R.S. *et al.* (2021) Alcohol-and-HIV-Induced Lysosomal Dysfunction Regulates Extracellular Vesicles Secretion in Vitro and in Liver-Humanized Mice. *Biology*. 10(1):29. doi. org/10.3390/biology10010029. <https://www.mdpi.com/2079-7737/10/1/29>

"As these vesicles contained EVs of mouse and human origin, we utilized a commercial human-specific pan-exosome isolation kit (Miltenyi Biotec, San Francisco, CA, USA; Cat 130-110-912), as per manufacturer's guidelines. The kit was based on the immunomagnetic separation of EVs markers, CD9, CD63, and CD81 expressed on human-specific EVs."

**Keywords:** serum, cell culture supernatant, next-generation RNA sequencing, exosome isolation kit

Mussack V. *et al.* (2019) Comparing small urinary extracellular vesicle purification methods with a view to RNA sequencing- Enabling robust and non-invasive biomarker research. *Biomol Detect Quantif*. 17:100089. doi:10.1016/j.bdq.2019.100089. <https://pubmed.ncbi.nlm.nih.gov/31194192/>

"The purest vesicles might be obtained by the positive selection via immunoaffinity (B), which enriched enough Alix+/ Hsp70-/TSG101+/Syntenin+/EPCAM-/CD63+/CD81+/CD9+-uEVs to perform small RNA-Seq and indicated a high content of miRNAs."

**Keywords:** urinary exosomes, small RNA sequencing, exosome isolation kit

Ando, W. *et al.* (2019) Novel breast cancer screening: combined expression of miR-21 and MMP-1 in urinary exosomes detects 95% of breast cancer without metastasis. *Sci Rep*. 9(1):13595. doi.org/10.1038/s41598-019-50084-5. <https://www.nature.com/articles/s41598-019-50084-5>

"Isolation and confirmation of urinary exosomes. Urinary exosomes isolated with a Miltenyi Biotec isolation kit (Bergisch Gladbach, Germany) were confirmed to be exosomes by western blotting with anti-CD63 antibody (Fig. 1A) and the size of isolated exosomes was determined by electron microscopy (Fig. 1B)."

**Keywords:** urinary exosomes, biomarkers, western blotting, electron microscopy, exosome isolation kit

## Comprehensive analysis of exosome surface proteins by flow cytometry

Martin-Jaular, L. *et al.* (2021) Unbiased proteomic profiling of host cell extracellular vesicle composition and dynamics upon HIV-1 infection. *EMBO J.* 40(8):e105492. doi:10.15252/embj.2020105492.

<https://pubmed.ncbi.nlm.nih.gov/33709510/>

"EVs isolated from Jurkat CCM (Fig 3B) and primary CD4<sup>+</sup> T CCM (Fig EV2B) by SEC were subjected to bead-based multiplex analysis by flow cytometry (MACSPlex Exosome Kit, human, Miltenyi). Samples were processed according to manufacturer's instructions, with 3 detection antibodies used separately. [...]  $2 \times 10^9$  EVs were diluted with MACSPlex buffer to a final volume of 120, and 10  $\mu$ L of MACSPlex Exosome Capture Beads was added. [...] After washing, detection antibodies (APC-conjugated anti-CD81 or anti-CD63 [included in the kit] or 5  $\mu$ L of anti-CD3E [Miltenyi, 130-113-697]) were incubated for 1 h at RT. [...] 100K pellets from control cells, SERINC3 KD cells and control cells after NL4-3 EGFP-Nef<sup>+</sup> infection (Fig 6G) were analysed by the MACSPlex Exosome Kit as above, but with detection by a mix of APC-conjugated anti-CD9/CD81/CD63 antibodies provided by the manufacturer, and including a fixation step with 4% PFA for 1 h to inactivate the virus.  $3-5 \times 10^8$  fixed EVs and 15  $\mu$ L of MACSPlex Exosome Capture Beads were used. Flow cytometric analysis was performed with a MACSQuant Analyzer 10 [...]."

**Keywords:** conditioned medium, T cells, HIV, exosome isolation kit, MACSPlex Exosome Kit, detection antibodies, MACSQuant Analyzer 10

Štok, U. *et al.* (2020) Characterization of plasma-derived small extracellular vesicles indicates ongoing endothelial and platelet activation in patients with thrombotic antiphospholipid syndrome. *Cells.* 9(5):1211. doi:10.3390/cells9051211.

<https://www.mdpi.com/2073-4409/9/5/1211>

"We used the MACSPlex Exosome Kit, which allows detection of 37 membrane surface epitopes [...] and included two isotype controls (mIgG1 and REA), corresponding to the antibodies used. [...] Briefly, sEVs-CD63 magnetic bead complexes were incubated with 15  $\mu$ L of MACSPlex Capture Beads and incubated overnight protected from light on an orbital shaker at 450 rpm at RT. [...] APC signal intensity in each of the 39 specific bead populations was measured on MACSQuant<sup>®</sup> Analyzer 10 [...]."

**Keywords:** plasma, antiphospholipid syndrome, CD63 exosome isolation kit, MACSPlex Exosome Kit, MACSQuant Analyzer 10

Görgens, A. *et al.* (2019) Optimisation of imaging flow cytometry for the analysis of single extracellular vesicles by using fluorescence-tagged vesicles as biological reference material. *J Extracell Vesicles.* 8(1):1587567. doi:10.1080/20013078.2019.1587567.

<https://pubmed.ncbi.nlm.nih.gov/30949308/>

"Conditioned medium (CM) samples were then filtered through 0.22  $\mu$ m filters and subjected to flow cytometric bead-based multiplex sEV analysis (MACSPlex Exosome Kit, human, Miltenyi Biotec) as described previously. [...] Next, the samples were [...] analysed by flow cytometry with a MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec)."

**Keywords:** conditioned medium, THP-1 cells, labelled EVs, GFP, MACSPlex Exosome Kit, MACSQuant Analyzer 10



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