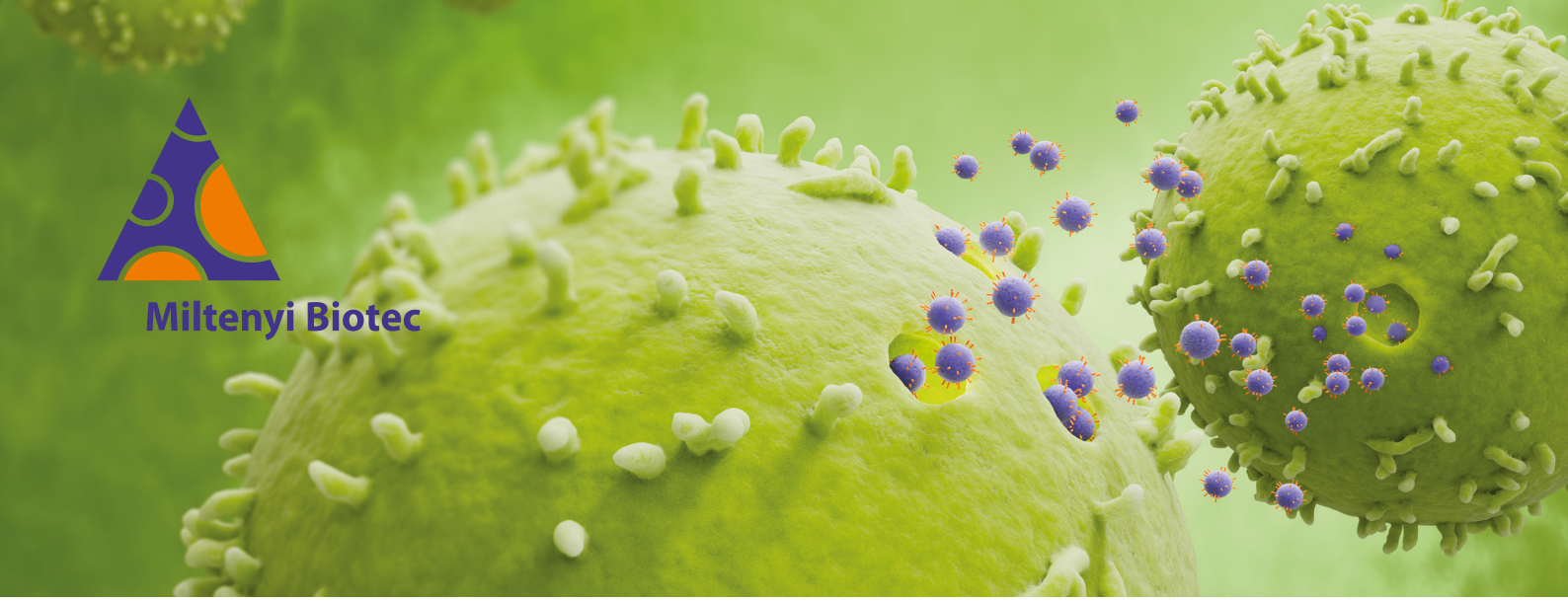

Miltenyi Biotec

Selected references

Comprehensive extracellular vesicle analysis by flow cytometry with the MACSPlex Exosome Kit, human

Extracellular vesicles (EVs), including exosomes, are secreted by virtually every cell type and can be found in all body fluids. They carry a variety of crucial molecules and are involved in many normal and pathological processes. However, EV analysis is challenging due to their small size and heterogeneous nature. Reliable and reproducible results are therefore only achievable when methods are carefully standardized. The selected references below show that the MACSPlex Exosome Kit, human represents a robust and semi-quantitative method for the simultaneous flow-cytometric analysis of 37 potential EV surface markers in a single sample.

Vasconcelos, M. H. *et al.* (2018) Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles. 7(1):1535750.
https://doi.org/10.1080/20013078.2018.1535750

"Numerous multiplexing approaches have been developed to analyse simultaneously the presence of a pre-designated set of surface protein markers on EVs. For instance, one platform uses flow cytometry after capture on an array of 37 beads, each bearing a specific antibody […]."

Keywords: Guidelines, standardization, reproducibility, flow cytometry, multiplexing.

Body fluid-derived EVs

Albino, D. *et al.* (2021) Circulating extracellular vesicles release oncogenic miR-424 in experimental models and patients with aggressive prostate cancer. Commun. Biol. 4(1):119.
https://doi.org/10.1038/s42003-020-01642-5

"An aliquot of 60 µL of sample underwent bead-based multiplex EVs capture and analysis by flow cytometry (FC), using MACSPlex human Exosome Kit (Miltenyi Biotec; Bergisch Gladbach, Germany), according to manufacturer's instructions. […] Median fluorescence intensity (MFI) was evaluated for each capture beads subsets and corrected by subtracting the respective MFI of blank control and normalized by the mean MFI of CD9, CD63, and CD81."

Keywords: Plasma, prostate cancer, miRNAs, MACSQuant® Analyzer 10

Vacchi, E. *et al.* (2021) Profiling inflammatory extracellular vesicles in plasma and cerebrospinal fluid: An optimized diagnostic model for Parkinson's disease. Biomedicines. 9(3):230.
https://doi.org/10.3390/biomedicines9030230

"In total, 60 µL of plasma and 30 µL of ultracentrifuged CSF were added to the MACSPlex Buffer solution (final volume 120 µL) and analyzed with MACSQuant Analyzer-10 flow cytometer (Miltenyi, Bergisch Gladbach, Germany). […] Median fluorescence intensity (MFI) for each EV surface marker was normalized by the mean MFI for specific EV markers (CD9, CD63, and CD81). All analyses were based on normalized MFI (nMFI) values. Samples were analyzed blind to the clinical diagnosis."

Keywords: Plasma, cerebrospinal fluid (CSF), biomarkers, Parkinson's disease, MACSQuant Analyzer 10

Forte, D. *et al.* (2021) Distinct profile of CD34+ cells and plasma-derived extracellular vesicles from triple-negative patients with Myelofibrosis reveals potential markers of aggressive disease. J. Exp. Clin. Cancer Res. 40(1):49. *https://doi.org/10.1186/s13046-020-01776-8*

"To phenotype EVs isolated from patients/HD, the MACSPlex Exosome Kit (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) was utilized. It provides the detection of 37 surface epitopes plus 2 isotype controls as for manufacturer's instructions. This approach allows semi-quantitative analysis of differential surface epitopes. The proportion of megakaryocyte (MK)- and platelets (PLT)-EVs in the isolated EVs samples was analyzed by flow cytometry as previously described […]."

Keywords: Plasma, myelofibrosis, microRNAs

Salvi, S. *et al.* (2021) Detection and investigation of extracellular vesicles in serum and urine supernatant of prostate cancer patients. Diagnostics (Basel). 11(3):466. *https://doi.org/10.3390/diagnostics11030466*

"The MACSPlex Exosome Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) allows the detection of 37 exosomal surface epitopes […]. The MACSPlex Exosome Detection Reagents for CD9, CD81, and CD63 were used to label the captured exosomes. Briefly, 6 µL and 80 µL of EVs from serum and urinary supernatant, respectively, were added to 114 µL and 40 µL of MACSPlex buffer, respectively, to obtain a final reaction volume of 120 µL. All samples were processed following the manufacturer's instructions. […] To avoid non-specific signals, from the raw median fluorescence intensity (MFI) of each marker was subtracted the MFI of the negative/blank control used in the same run experiment."

Keywords: Urine, serum, prostate cancer, liquid biomarker

d'Alessandro, M. *et al.* (2021) Extracellular vesicle surface signatures in IPF patients: A multiplex bead-based flow cytometry approach. Cells. 10(5):1045. *https://doi.org/10.3390/cells10051045*

"MACSPlex analysis was performed using the MACSPlex Exosome Kit, human (Miltenyi Biotec, Bergisch-Gladbach, Germany), which detects 37 exosomal surface epitopes plus two isotype controls. After indirect determination of the EV concentration by quantifying the protein concentration, EV-containing samples were processed as follows: isolated EVs (4–20 µg protein) from each sample were diluted with MACSPlex buffer (MPB) to a final volume of 120 µL and loaded into 1.5-mL tubes with 15 µL MACSPlex Exosome Capture Beads. […] For further analysis, background values of the control sample (PBS) of each run were subtracted from the sample values. Exosomal surface epitope concentrations were obtained from the ratio of beads + EVs + Ab to the corresponding controls (capture beads + Ab)."

Keywords: Serum, idiopathic pulmonary fibrosis, biomarkers, prognosis

Conzelmann, C. *et al.* (2020) Salivary extracellular vesicles inhibit Zika virus but not SARS-CoV-2 infection. J. Extracell. Vesicles. 9(1):1808281. *https://doi.org/10.1080/20013078.2020.1808281*

"Purified EVs were subjected to bead-based multiplex EV analysis (MACSPlex Exosome Kit, human, Miltenyi Biotec) as previously described. Briefly, EVs purified by TFF/BE-SEC were diluted at input doses of 1×10^9 NTA-quantified particles per assay in a total of 60 µl MACSplex buffer and incubated overnight with 3 µl MACSPlex Exosome Capture Beads on an orbital shaker at 450 rpm at room temperature. […] For counter staining of captured EVs, a mixture of individual APC-conjugated anti-CD9, anti-CD63 or anti-CD81 detection antibodies or a mixture of all three antibodies (supplied in the MACSPlex kit, 4 µl each) were added to each well […]."

Keywords: Saliva, urine, Zika virus, SARS-CoV-2, MACSQuant Analyzer 10

Martínez-González, E. *et al.* (2020) Comparison of methods and characterization of small RNAs from plasma extracellular vesicles of HIV/HCV coinfected patients. Sci. Rep. 10(1):11140. *https://doi.org/10.1038/s41598-020-67935-1*

"Pooled plasma (clarified at 10.000 rpm 20 min at 4 °C and filtered with a 0.22 µm filter) was analyzed by bead-based multiplex EV analysis by flow cytometry (MACSPlex Exosome Kit, human, Miltenyi Biotec), following manufacturer instructions. […] Sandwich complexes were analyzed based on its fluorescence characteristics in a MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec), […]. Median fluorescence signal intensity (MFI) for all 39 capture bead subsets were background corrected by subtracting respective MFI values from the negative control."

Keywords: Plasma, hepatitis C virus, HIV infections, smRNAs, 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. *https://doi.org/10.3390/cells9051211*

"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 µL 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 a MACSQuant® Analyzer 10 […]."

Keywords: Plasma, antiphospholipid syndrome, CD63 Exosome Isolation Kit, MACSQuant Analyzer 10

Cell culture supernatant-derived EVs

Pomatto, M. *et al.* (2021) Differential therapeutic effect of extracellular vesicles derived by bone marrow and adipose mesenchymal stem cells on wound healing of diabetic ulcers and correlation to their cargoes. Int. J. Mol. Sci. 22(8):3851. *https://doi.org/10.3390/ijms22083851*

"For analysis using the human cytofluorimetric bead-based MACSPlex exosome kit (Miltenyi Biotec, Bergisch Gladbach, Germany) the manufacturer's protocol was followed. […] During acquisition, the median fluorescence intensity (MFI) for all 39 exosomal markers were corrected for medium background and gated based on their respective fluorescence intensity as per manufacturer's instructions."

Keywords: Cell culture supernatant, mesenchymal stem cells (MSCs), diabetes

Kaspi, H. *et al.* (2021) MSC-NTF (NurOwn®) exosomes: a novel therapeutic modality in the mouse LPS-induced ARDS model. Stem Cell. Res. Ther. 12(72).
https://doi.org/10.1186/s13287-021-02143-w

"Characterization of EV membranal markers was performed with the MACSPlex exosomes kit (Miltenyi) with 7.5 × 10^8 EVs per sample."

Keywords: Conditioned medium, MSCs, acute respiratory distress syndrome, COVID-19

Cavallo, C. *et al.* (2021) Small extracellular vesicles from adipose derived stromal cells significantly attenuate *in vitro* the NF-κB dependent inflammatory/catabolic environment of osteoarthritis. Sci. Rep. 11(1):1053.
https://doi.org/10.1038/s41598-020-80032-7

"To characterize sEV surface epitopes, 5 µg of sEV were analyzed using the MACSPlex Exosome Kit (MILTENYI BIOTEC) that allows the detection of 37 surface markers and two isotype controls. sEV were incubated with MACSPlex Exosome Capture Beads and with MACSPlex Exosome Detection Reagent CD9, CD63, and CD81 for 1 h at RT."

Keywords: Conditioned medium, adipose-derived stromal cells (ADSC), osteoarthritis

Shin, K. O. *et al.* (2020) Exosomes from human adipose tissue-derived mesenchymal stem cells promote epidermal barrier repair by inducing de novo synthesis of ceramides in atopic dermatitis. Cells. 9(3):680.
https://doi.org/10.3390/cells9030680

"The isolated ASC-exosomes were captured and labeled with the MACSPlex Exosome kit, human (Miltenyi Biotec, Bergish Gladbach, Germany) according to the manufacturer's instructions."

Keywords: Conditioned medium, adipose tissue-derived MSCs (ASCs), skin disease

Kholia, S. *et al.* (2020) Mesenchymal stem cell derived extracellular vesicles ameliorate kidney injury in aristolochic acid ephropathy. Front. Cell Dev. Biol. 24(8):188.
https://doi.org/10.3389/fcell.2020.00188

"The expression of surface markers was evaluated using the human cytofluorimetric bead-based MACSPlex exosome kit (Miltenyi Biotec, Germany) according to manufacturer's protocol."

Keywords: Cell culture supernatant, MSCs, chronic kidney disease

Almeria, C. *et al.* (2019) Hypoxia conditioned mesenchymal stem cell-derived extracellular vesicles induce increased vascular tube formation *in vitro*. Front. Bioeng. Biotechnol. 7:292.
https://doi.org/10.3389/fbioe.2019.00292

"MSCs were seeded at 3,000 cells/cm^2 in passage 2 and cultivated for 72 h. The cell culture supernatants were subjected to bead-based multiplex EV analysis by flow cytometry (MACSPlex Exosome Kit, human; Miltenyi Biotec, Bergisch Gladbach, Germany). To obtain samples for EV characterization, supernatants were pre-cleared according to manufacturer's recommendation."

Keywords: Cell culture supernatant, MSCs, MACSQuant Analyzer 10

Collino, F. *et al.* (2020) Extracellular vesicles derived from induced pluripotent stem cells promote renoprotection in acute kidney injury model. Cells. 9(2):453.
https://doi.org/10.3390/cells9020453

"Further characterization of iPSC-EV markers was performed using the bead-based multiplex exosome flow cytometry assay (MACSPlex Exosome Kit human, Miltenyi Biotec, Bergisch Gladbach, Germany). Shortly after, samples were diluted with the MACSPlex buffer to a final concentration of 4–20 µg of protein and a final volume of 120 µL. A total of 15 µL of the MACSPlex Exosome Capture beads, which contain 39 different antibody-coated beads, and 15 µL of MACSPlex Exosome Detection Reagent cocktail, was added."

Keywords: Cell culture supernatant, iPSCs, kidney disease

Calleri, A. *et al.* (2021) Protective effects of human liver stem cell-derived extracellular vesicles in a mouse model of hepatic ischemia-reperfusion injury. Stem Cell. Rev. Rep. 17(2):459-470.
https://doi.org/10.1007/s12015-020-10078-7

"HLSC-EV were characterized by bead-based multiplex analysis by flow cytometry (MACSPlex Exosome Kit, human, Miltenyi Biotec). Briefly, 1 × 10^9 EV were diluted with MACSPlex buffer (MPB) to a final volume of 120 µL and loaded into a 1.5-mL tube."

Keywords: Cell culture supernatant, HLSC, hepatic inflammation

Morandi, F. *et al.* (2020) Human amnion epithelial cells impair T cell proliferation: The role of HLA-G and HLA-E molecules. Cells. 9(9):2123.
https://doi.org/10.3390/cells9092123

"The multiplex-bead based analysis of surface markers was performed on ssEV using the MACSPlex Exosome kit (MiltenyiBiotec) by using allophycocyanin (APC)-conjugated pan-tetraspaninantibodies included in the kit for detection (CD9/CD63/CD81), as previously described."

Keywords: Preconditioned supernatant, amnion epithelial cells, small-size EV, MACSQuant Analyzer 10

Priglinger, E. *et al.* (2020) SVF-derived extracellular vesicles carry characteristic miRNAs in lipedema. Sci. Rep. 10(1):7211.
https://doi.org/10.1038/s41598-020-64215-w

"The MACSPlexExosome Kit (MiltenyiBiotec, Bergisch Gladbach, Germany) is a bead-based multiplexed FACS-based assay for the analysis of surface markers present on EVs. We have used the MACSPlex kit according to the manufacturer's instruction and following a validated standard operating procedure with 5 × 10^7 to 5 × 10^8 particles as input."

Keywords: Conditioned medium, stromal vascular fraction (SVF), lipedema, miRNA, biomarker

Lopatina, T. *et al.* (2020) Targeting IL-3Rα on tumor-derived endothelial cells blunts metastatic spread of triple-negative breast cancer via extracellular vesicle reprogramming. Oncogenesis. 9(10):90.
https://doi.org/10.1038/s41389-020-00274-y

"Moreover, EV flow cytometry analysis was performed using the MACSPlex Exosome Kit (human, Miltenyi Biotec), following the manufacturer's protocol."

Keywords: Conditioned medium, tumor-endothelial cells, breast cancer

Custom detection antibodies

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. *https://doi.org/10.15252/embj.2020105492*

"EVs isolated from Jurkat CCM (Fig 3B) and primary CD4⁺ 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 manufacture's instructions, with 3 detection antibodies used separately. […] 2 × 10^9 EVs were diluted with MACSPlex buffer to a final volume of 120, and 10 µl of MACSPlex Exosome Capture Beads was added. […] After washing, detection antibodies (APC–conjugated anti–CD81 or anti–CD63 [included in the kit] or 5 µl 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⁺ 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 × 10^8 fixed EVs and 15 µl 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, detection antibodies, MACSQuant Analyzer 10

Warnecke, A. *et al.* (2020) Extracellular vesicles from human multipotent stromal cells protect against hearing loss after noise trauma *in vivo*. Clin. Transl. Med. 10(8):e262. *https://doi.org/10.1002/ctm2.262*

"The bead-based multiplexed FACS–based MACSPlex Exosome Kit (Miltenyi Biotec) is an assay for the analysis of surface markers present on EVs. To characterize the various MSC–EV preparations, we used the MACSPlex kit according to the manufacturer's instructions and following a validated standard operating procedure with 5 × 10^7 to 5 × 10^8 total particles as input. For additional CD73 analyses, an anti–CD73–BV421 antibody (BD Biosciences) was used. Data normalization was directed toward CD9/CD63/CD81 APC signal."

Keywords: Cell culture supernatant, MSCs, GMP, detection antibodies

Bruno, S. *et al.* (2020) HLSC-Derived Extracellular Vesicles Attenuate Liver Fibrosis and Inflammation in a Murine Model of Non-alcoholic Steatohepatitis. Mol. Ther. 28(2):479-489. *https://doi.org/10.1016/j.ymthe.2019.10.016*

"EV-HLSCs were characterized by cytofluorimetric analysis. Different EV-HLSC preparations (n = 3) were subjected to bead-based multiplex analysis by flow cytometry (MACSPlex Exosome Kit, human, Miltenyi Biotec). […] In this study, we mostly used a mixture of all three antibodies (pan-tetraspanin) in order to cover most EVs being present in the samples. In selected experiments, we used 5 µL APC-conjugated anti-CD29 (Miltenyi Biotec) antibody instead of the anti-tetraspanin antibodies."

Keywords: Cell culture supernatant, HLSC, chronic liver disease, detection antibodies

Phenotyping of labelled EVs

Gupta, D. *et al.* (2020) Quantification of extracellular vesicles *in vitro* and *in vivo* using sensitive bioluminescence imaging. J. Extracell. Vesicles.9(1): 1800222. *https://doi.org/10.1080/20013078.2020.1800222*

"Conditioned media samples were subjected to bead-based multiplex EV analysis by flow cytometry (MACSPlex Exosome Kit, human, Miltenyi Biotec) as described previously. […] Flow cytometric analysis was performed with a MACSQuant Analyser 10 flow cytometer by using the built-in 96-well plate reader."

Keywords: Conditioned medium, HEK-293T, EV labelling, luciferase, 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. *https://doi.org/10.1080/20013078.2019.1587567*

"Conditioned medium (CM) samples were then filtered through 0.22 µm 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, MACSQuant Analyzer 10

130-130-534.01