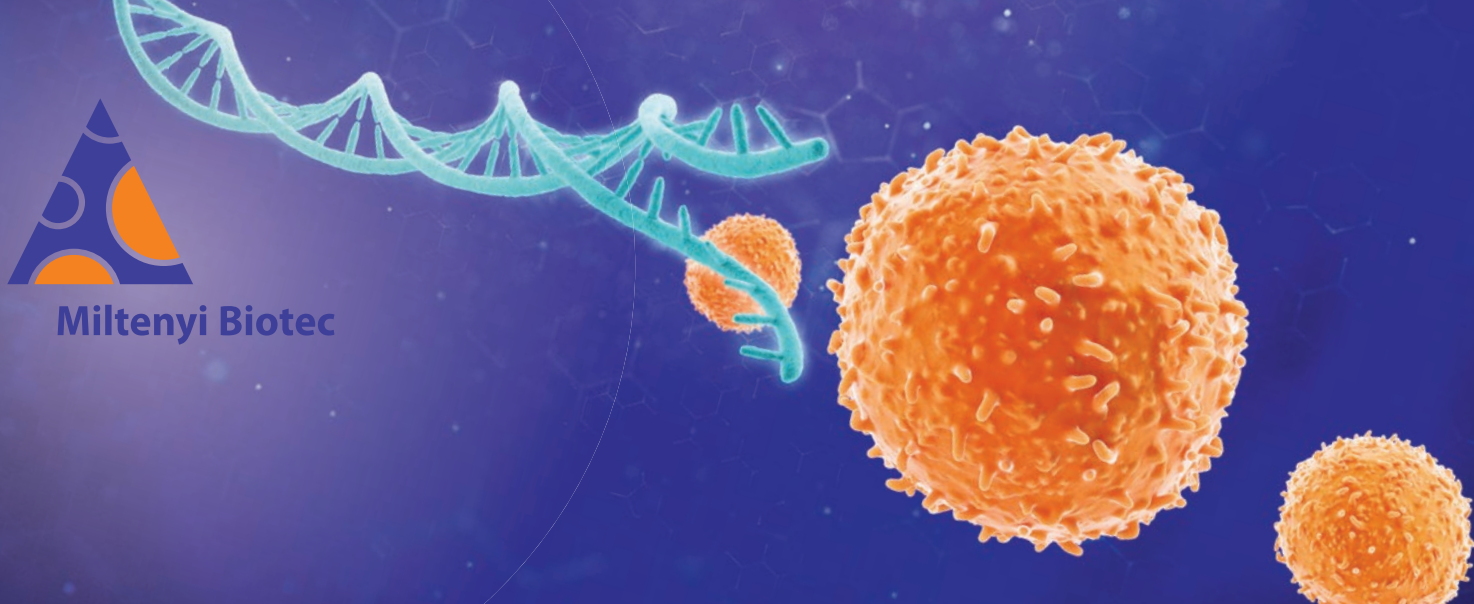




**Miltenyi Biotec**

## Reference list

# Automated cell separation for downstream omics applications

Whether you do single cell or bulk analysis for your genomics, epigenetics or transcriptomics research, the purity of cells is of vital importance. Using our technology and cell separation solutions can open new avenues of opportunity for omics studies. Reliable and publishable results are easily achievable when methods are standardized, and target cells are pure. The selected references below show the obtainable success when using MACS® Cell Separation. Our MicroBead kits, autoMACS® Pro Separator, and other products are indispensable for excellent analyses and downstream applications.

## Selected references on human-derived material

Garrett-Bakelman, F. E. *et al.* (2019) The NASA twins study: A multidimensional analysis of a year-long human spaceflight. *Science*. 364,6436 eaau8650. doi:10.1126/science.aau8650.

*"Data were generated across human and microbial cells, including isolations of stool, saliva, skin, urine, blood, plasma, PBMCs, and immune cells that are CD4<sup>+</sup>, CD8<sup>+</sup>, and CD19<sup>+</sup> enriched and lymphocyte-depleted (LD), from autoMACS magnetic bead separation and validated by FACS."*

**Keywords: Whole blood, CD4<sup>+</sup> cells, CD8<sup>+</sup> cells, CD19<sup>+</sup> cells, sequential positive selection, autoMACS Pro Separator, Next-Generation Sequencing**

Sumimoto, H. *et al.* (2020) Detection of neoantigen-reactive T cell clones based on the clonal expansion using next-generation sequencing of T cell receptor  $\beta$  complementarity-determining region 3. *J Immunol Methods*. 476:112679. doi:10.1016/j.jim.2019.112679.

*"CD3<sup>+</sup> T cells were used as responder cells after isolation from characterized cryopreserved human peripheral blood mononuclear cells (PBMCs), derived from an HLA-A\*02:01-positive donor, [...], using CD3 MicroBeads (Miltenyi Biotec, # 130-050-101) and an autoMACS Pro Separator (Miltenyi Biotec). [...] CD8<sup>+</sup> T cells were isolated from PBMCs or cultured CD3<sup>+</sup> T cells using CD8 MicroBeads (Miltenyi Biotec, Germany; # 130-045-201) and an autoMACS Pro Separator (Miltenyi Biotec)."*

**Keywords: Cryopreserved PBMCs, CD3<sup>+</sup> cells, CD8<sup>+</sup> cells, positive selection, autoMACS Pro Separator, System and Ion PGM™ System**

Palmirotta, R. *et al.* (2017) Next-generation sequencing (NGS) analysis on single circulating tumor cells (CTCs) with no need of whole-genome amplification (WGA). *Cancer Genomics Proteomics*. 14.3:173-179. doi:10.21873/cgp.20029.

*"The spiked blood sample was processed by Ficoll® (Sigma) gradient and depleted of CD45<sup>+</sup> and Glycophorin<sup>+</sup> cells by immunomagnetic sorting procedure using an autoMACS Pro Separator (Miltenyi). The negative selected cells were stained with anti-Melan-A phycoerythrin (PE) and anti-CD45 allophycocyanin (APC) (Miltenyi) conjugated antibodies, whereas nuclei were displayed with Hoechst 33342 after fixation and permeabilization. In particular, anti-CD45 antibody was used to exclude contaminating leucocytes."*

**Keywords: Whole blood, CD45<sup>+</sup> cells, depletion, autoMACS Pro Separator, Ion Torrent PGM™ System**

De la Rosa, I. A. *et al.* (2020) Impaired microRNA processing in neutrophils from rheumatoid arthritis patients confers their pathogenic profile. Modulation by biological therapies. *Haematologica*. 105.9:2250-2261. doi:10.3324/haematol.2018.205047.

*"Neutrophils from PB of HD and paired SF and PB samples of RA patients were isolated (after centrifugation to obtain buffy coat and osmotic lysis of the pellet) by immunomagnetic positive selection with human anti-CD15 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) using an autoMACS Pro Separator (Miltenyi Biotec)."*

**Keywords: Whole blood, CD15<sup>+</sup> cells, positive selection, autoMACS Pro Separator, NanoString nCounter Technology**

Krusche, J. *et al.* (2019) TNF- $\alpha$ -induced protein 3 is a key player in childhood asthma development and environment-mediated protection. *J Allergy Clin Immunol Pract*. 144.6:1684-1696.e12. doi:10.1016/j.jaci.2019.07.029.

"DCs were separated from unstimulated and German dust-stimulated PBMCs of CLARA/CLAUS children by using an autoMACS Pro Separator (Miltenyi Biotec, Bergisch Gladbach, Germany) with the DC isolation kit."

**Keywords: PBMCs, dendritic cells, untouched selection, autoMACS Pro Separator, NanoString nCounter Technology**

Ong, L. T. C. et al. (2020) Regulation of the methylome in differentiation from adult stem cells may underpin vitamin D risk in MS. *Genes Immun.* 21.5:335-347. doi:10.1038/s41435-020-00114-4.

"Approximately 100 mL of whole blood was drawn from each of 13 healthy individuals into tubes containing EDTA. Peripheral blood mononuclear cells (PBMCs) were then obtained by density gradient centrifugation and divided into multiple aliquots (1×10<sup>7</sup> PBMCs for CD14<sup>+</sup> and CD56<sup>+</sup>, the remainder for CD34<sup>+</sup>). Parallel positive selection was performed using CD14 MicroBeads, CD34 MicroBead Kit Ultrapure and CD56 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) for each respective cell subset with the autoMACS Pro Separator (Miltenyi Biotec, Bergisch Gladbach, Germany) as per manufacturer's instructions. An aliquot of purified cells was set aside for assessment of purity by flow cytometry. Samples used for downstream analysis demonstrated mean purities of 96% for CD14<sup>+</sup>, 93% for CD34<sup>+</sup> and 96% for CD56<sup>+</sup> cells. Purified cells were subjected to two subsequent washes with DPBS before storage at -80 °C."

**Keywords: Whole blood, CD14<sup>+</sup> cells, CD34<sup>+</sup> cells, CD56<sup>+</sup> cells, positive selection, autoMACS Pro Separator, Illumina HiSeq 2500**

Podojil, J. R. et al. (2020) Antibody targeting of B7-H4 enhances the immune response in urothelial carcinoma. *Oncoimmunology.* 9.1:1744897. doi:10.1080/2162402X.2020.1744897.

"PBMCs from healthy donors (n = 5) (LifeSource; Evanston, IL) were collected and CD14<sup>+</sup> monocytes were purified via autoMACS magnetic bead cell separation technology. The CD14-fractions were collected, and CD4<sup>+</sup> and CD8<sup>+</sup> T cells purified via autoMACS magnetic bead cell separation technology. The autologous CD4<sup>+</sup> and CD8<sup>+</sup> T cells were cryopreserved for use in the monocyte/T cell co-cultures."

**Keywords: PBMCs, CD14<sup>+</sup> cells, untouched selection, CD4<sup>+</sup> cells, CD8<sup>+</sup> cells, positive selection, autoMACS Pro Separator, 10x Genomics Chromium**

Voigt, A. P. et al. (2019) Single-cell transcriptomics of the human retinal pigment epithelium and choroid in health and macular degeneration. *Proc Natl Acad Sci U S A.* 116.48:24100-24107. doi:10.1073/pnas.1914143116.

"Cryopreserved cells from donors 4 to 7 were rapidly thawed before pre-enrichment for endothelial cells. Cells were incubated for 15 min with antiCD31 MicroBeads (Miltenyi Biotec) according to the manufacturer's instructions before automated autoMACS magnetic separation (Miltenyi Biotec) of the CD31<sup>+</sup> cell fraction. The positive cell fraction was passed 2 times through a 40 µm filter to remove aggregates. Single cells were immediately barcoded before library sequencing as described above. Cells with unique gene counts fewer than 500 were eliminated, and cells with more than 7,000 unique genes per cell were removed to eliminate potential doublets."

**Keywords: Cryopreserved cells, CD31<sup>+</sup> cells, positive selection, autoMACS Pro Separator, Illumina HiSeq 4000**

Kim, D. et al. (2020) Somatic mTOR mutation in clonally expanded T lymphocytes associated with chronic graft versus host disease. *Nat Commun.* 11.1 2246. doi:10.1038/s41467-020-16115-w.

"Mononuclear cells (MNCs) were separated from whole blood using Ficoll®. The separated MNCs were then labeled with either CD4<sup>+</sup> or CD8<sup>+</sup> magnetic beads (Miltenyi Biotec) and sorted by autoMACS Pro Separator (Miltenyi Biotec) according to the manufacturer's protocol. The purity of sorted fractions was evaluated by flow cytometry and confirmed to be >98%."

**Keywords: Whole blood, CD4<sup>+</sup> cells, CD8<sup>+</sup> cells, positive selection, autoMACS Pro Separator, Illumina HiSeq 2500**

Laggner, M. et al. (2020) Therapeutic potential of lipids obtained from γ-irradiated PBMCs in dendritic cell-mediated skin inflammation. *EBioMedicine.* 55:102774. doi:10.1016/j.ebiom.2020.102774.

"For transcriptomics, CD14<sup>+</sup> monocytes were enriched from PBMCs by magnetic sorting using human CD14 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) and autoMACS Pro Separator (Miltenyi Biotec) as suggested by the manufacturer. [...] To study DC-mediated priming of T cells into T helper cell subsets, naïve CD4<sup>+</sup> T cells were enriched from PBMCs using human naïve CD4<sup>+</sup> T Cell Isolation Kit II (Miltenyi Biotec) as suggested by the manufacturer."

**Keywords: PBMCs, CD14<sup>+</sup> cells, positive selection, CD4<sup>+</sup> cells, untouched selection, autoMACS Pro Separator, 10x Genomics Chromium**

Canu, G. et al. (2020) Analysis of endothelial-to-haematopoietic transition at the single cell level identifies cell cycle regulation as a driver of differentiation. *Genome Biol.* 21.1:157. doi:10.1186/s13059-020-02058-4.

"Two volumes of blood dilution were transferred in 50 mL tubes on a layer of 1 volume of Ficoll®. After centrifugation for 15 min at 800 g, the resulting layer of mononuclear cells was carefully removed, [...] Cells were then processed for CD34<sup>+</sup> enrichment using labelling with magnetic beads. Briefly, cells were resuspended in the same PBS/EDTA/human serum albumin buffer, added with 50 µL/108 cells of FcR blocking reagent (Miltenyi Biotec) and 50 µL/108 cells of CD34 magnetic beads (Miltenyi Biotec), and incubated for 30 min at 4 °C. After incubation, cells were washed using the same buffer and processed with autoMACS Pro Separator to enrich for CD34<sup>+</sup> peripheral blood mononuclear cells."

**Keywords: Whole blood, CD34<sup>+</sup> cells, positive selection, autoMACS Pro Separator, 10x Genomics Chromium**

Papa, L. et al. (2020) Limited mitochondrial activity coupled with strong expression of CD34, CD90 and EPCR determines the functional fitness of ex vivo expanded human hematopoietic stem cells. *Front Cell Dev Biol.* 8:592348. doi:10.3389/fcell.2020.592348.

"Fresh umbilical cord bloods (UCBs) were purchased from the Placental Blood Program at the New York Blood Center. Mononuclear cells were isolated from UCBs by Ficoll® [...] followed by CD34<sup>+</sup> cell selection by immunomagnetic sorting using the CD34 Microbead kit (Miltenyi) and the autoMACS Pro Separator (Miltenyi) as previously described (Papa et al., 2019a)."

**Keywords: Umbilical cord blood, CD34<sup>+</sup> cells, positive selection, autoMACS Pro Separator, 10x Genomics Chromium**

Koczan, D. et al. (2018) Microarray data of transcriptome shifts in blood cell subsets during S1P receptor modulator therapy. *Sci Data.* 5:180145. doi:10.1038/sdata.2018.145.

"The blood samples were taken in the routine setting before the start of fingolimod therapy (baseline), the next day before the second dose (24-hour time point) as well as at a follow-up visit after the first 3 months of treatment. [...] From each sample, 5 vials with 4 mL EDTA blood were prepared for the magnetic cell sorting. Each vial was mixed with 200 µL Whole Blood MicroBeads that

were conjugated to antibodies against the cell surface markers CD4 (order no. 130-090-877), CD8 (130-090-878), CD14 (130-090-879), CD19 (130-090-880) and CD56 (130-090-875), respectively (Miltenyi Biotec, Bergisch Gladbach, Germany). The samples were then incubated for 15 min at 4 °C. The cell separation was done using a Miltenyi Biotec autoMACS Pro Separator according to the manufacturer's instructions. By this means, magnetically labeled cells were eluted from the columns as positively selected cell fractions."

**Keywords:** Whole blood, CD4<sup>+</sup> cells, CD8<sup>+</sup> cells, CD14<sup>+</sup> cells, CD19<sup>+</sup> cells, CD56<sup>+</sup> cells, StraightFrom positive selection, CD4<sup>+</sup> cells, untouched selection, autoMACS Pro Separator, Microarray

Rammensee, H. et al. (2019) A new synthetic toll-like receptor 1/2 ligand is an efficient adjuvant for peptide vaccination in a human volunteer. *J Immunother Cancer*. 7:1:307. doi:10.1186/s40425-019-0796-5.

"PBMCs were incubated with M-DC8 antibody containing hybridoma supernatant, labeled with rat anti-mouse IgM coupled to paramagnetic MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) and sorted (autoMACS Pro Separator; Miltenyi). CD56<sup>+</sup> CD3<sup>neg</sup> NK cells and CD3<sup>+</sup> CD4<sup>+</sup> T cells were isolated from PBMCs by immunomagnetic depletion (Miltenyi). Purity of sorted cells >90% was confirmed by flow cytometry."

**Keywords:** Whole blood, CD56<sup>+</sup> CD3<sup>-</sup> NK cells, CD4<sup>+</sup> T cells, Slan (M-DC8)<sup>+</sup>, untouched selection, autoMACS Pro Separator, Microarray

Ruhrmann, S. et al. (2018) Hypermethylation of MIR21 in CD4<sup>+</sup> T cells from patients with relapsing-remitting multiple sclerosis associates with lower miRNA-21 levels and concomitant up-regulation of its target genes. *Mult Scler*. 24:10:1288-1300. doi:10.1177/1352458517721356.

"For discovery and validation cohorts, peripheral blood mononuclear cells (PBMCs) were isolated using a standard Ficoll® (GE Healthcare) and sodium citrate-containing preparation tubes (Becton Dickinson) procedures, respectively. Sorting of the CD4<sup>+</sup> T cells was performed on a MoFlo™ cell sorter (Beckman Coulter) and an autoMACS® Pro Separator (Miltenyi Biotec), respectively. Extraction of genomic DNA was carried out using GenElute Mammalian Genomic DNA Miniprep kit (SigmaAldrich). RNA was isolated using standard TRIzol protocol (Invitrogen) and Allprep Total RNA/DNA Kit (Qiagen)."

**Keywords:** Whole blood, CD4<sup>+</sup> cells, positive selection, autoMACS Pro Separator, Illumina HiSeq 2500

Elmentaite, R. et al. (2020) Single-cell sequencing of developing human gut reveals transcriptional links to childhood Crohn's disease. *Dev Cell*. 55:6:771-783.e5. doi:10.1016/j.devcel.2020.11.010.

"Fetal cells were either loaded for scRNAseq directly following sample processing or subjected to EPCAM selection to enrich epithelial cells. For enrichment, single cells were suspended in MACS modified solution (PBS with 0.5% BSA, 2 mM EDTA and 100 IU/mL DNaseI) and stained with EPCAM (CD326) magnetic MicroBeads (Miltenyi Biotec) according to the manufacturer's protocol. Enrichment was performed using an autoMACS Pro Separator. Either only EPCAM positive (PCW 6.7, 6.9, 10.2, 9.3) or both EPCAM positive and negative (PCW 9.9, 10.1, 10) fractions were processed using the 10x Genomics single-cell transcriptomics system. All paediatric single-cell suspensions were subjected to the MACS enrichment using the same protocol as described for fetal samples."

**Keywords:** Fetal cells, EPCAM (CD326) cells, positive selection, autoMACS Pro Separator, 10x Genomics Chromium

## Selected references on mouse-derived material

Aoki, H. et al. (2019) TCR repertoire analysis reveals mobilization of novel CD8<sup>+</sup> T cell clones into the cancer-immunity cycle following anti-CD4 antibody administration. *Front Immunol*. 9:3185. doi:10.3389/fimmu.2018.03185.

"Single-cell suspensions from the dLN, PBL, and tumor were prepared by enzymatic or mechanical dissociation of tissues. For the purification of CD8<sup>+</sup> T cells, CD4, and lineage (CD11b, CD19, NK1.1, and Ter119) positive cells were depleted using an autoMACS Pro Separator (Miltenyi Biotec, Bergisch Gladbach, Germany), and CD8<sup>+</sup> T cells or CD8<sup>+</sup> CD44<sup>hi</sup> T cells were then sorted. [...] TCR-seq libraries for next generation sequencing (NGS) were prepared from the mRNA of sorted T cell samples."

**Keywords:** Mouse tumor sample, CD4<sup>+</sup> cells, CD8<sup>+</sup> cells, CD11b<sup>+</sup> cells, CD19<sup>+</sup> cells, NK1.1<sup>+</sup> cells, Ter199<sup>+</sup> cells, depletion, autoMACS Pro Separator, Ion Proton next generation sequencer

Dick, S. A. et al. (2019) Self-renewing resident cardiac macrophages limit adverse remodeling following myocardial infarction. *Nat Immunol*. 20:1:29-39. doi:10.1038/s41590-018-0272-2.

"Mouse hearts were isolated and enzymatically digested, as previously described, with the inclusion of 1mM Flavopiridol in the digestion buffer. Digestions were stopped after 20 min and cells were processed into a single cell suspension on ice. Cells were stained with CD45 magnetic beads (Miltenyi Biotec) and subsequently fluorescently tagged antibodies against CD45, CD64 and CD11b. Hematopoietic cells were positively enriched using an autoMACS Pro Separator (Miltenyi Biotec) [...] followed by flow sorting."

**Keywords:** Mouse heart, CD45<sup>+</sup> cells, positive selection, autoMACS Pro Separator, 10x Genomics Chromium

Herrero, D. et al. (2018) Redox-dependent BMI1 activity drives in vivo adult cardiac progenitor cell differentiation. *Cell Death Differ*. 25:4:809-822. doi:10.1038/s41418-017-0022-2.

"For ChIP analysis, we used a Sca1<sup>+</sup>CD45<sup>-</sup>-enriched cardiac population. After Langendorff heart digestion, we depleted the hematopoietic lineage (CD45<sup>+</sup>) by immunomagnetic separation (autoMACS Pro Separator); the SCA1<sup>+</sup> fraction was purified by magnetic cell sorting using a lineage cell depletion kit and Sca1 MicroBeads (all from Miltenyi Biotec). Homeostasis and damaged Sca1<sup>+</sup>CD45<sup>-</sup> cells were isolated and immunoprecipitated synchronically to avoid procedural differences."

**Keywords:** Mouse heart, CD45<sup>+</sup> cells, depletion, Sca1<sup>+</sup> cells, positive selection, autoMACS Pro Separator, Illumina HiSeq 2500

Horikawa, N. et al. (2020) Anti-VEGF therapy resistance in ovarian cancer is caused by GM-CSF-induced myeloid-derived suppressor cell recruitment. *Br J Cancer*. 122:6:778-788. doi:10.1038/s41416-019-0725-x.

"To generate in vitro-induced myeloid cells, bone marrow cells were harvested from the femurs and tibias of naive B6 mice. CD11b<sup>+</sup> population was isolated using auto magnetic-activated cell sorting (autoMACS Pro Separator, Miltenyi Biotec) [...]. Carboxyfluorescein succinimidyl ester (CFSE) (10 µm) was added to the cell suspension (1×10<sup>7</sup> cells/mL) of T cells separated from splenocytes of a wild-type C57BL/6 mouse using mouse Pan T cell isolation kit (Miltenyi Biotec)."

**Keywords:** Mouse bone marrow, CD11b<sup>+</sup> cells, positive selection, Pan T cells, untouched selection, autoMACS Pro Separator, Microarray

Cippà, P. E. *et al.* (2019) A late B lymphocyte action in dysfunctional tissue repair following kidney injury and transplantation. *Nat Commun.* 10.1:1157. 11 doi:10.1038/s41467-019-09092-2.

*"To isolate kidney infiltrating lymphocytes the mouse was perfused with PBS until the kidney was visually blood-free. Then the kidney was removed, mechanically dissociated, and then incubated at 37 °C in digestion buffer (RPMI medium, 1 mg/mL type I collagenase, 10 µg/mL DNase, 10% FCS, 25 mM HEPES, penicillin and streptomycin). After 60 min, the tissue was filtered through 100 µm nylon mesh to remove remaining large fragments. CD45<sup>+</sup> cells were positively sorted by magnetic cell separation on an autoMACS Pro Separator with anti-mouse CD45 beads (Miltenyi Biotec, Cat# 130-053-301)."*

**Keywords: Mouse kidney, kidney infiltrating lymphocytes, CD45<sup>+</sup> cells, positive selection, autoMACS Pro Separator, Illumina HiSeq 2000**

Sugita, J. *et al.* (2021) Cardiac macrophages prevent sudden death during heart stress. *Nat Commun.* 12.1:1910. doi:10.1038/s41467-021-22178-0.

*"Mouse cardiomyocyte cultures were prepared from one- to three-day old male and female C57BL6/J mouse pups using a neonatal heart dissociation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). Cardiac macrophages were depleted from primary cultures of cardiomyocytes using anti-CD11b MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) with an autoMACS Pro Separator according to the manufacturer's instructions."*

**Keywords: Mouse heart, CD11b<sup>+</sup> cells, depletion, autoMACS Pro Separator, 10x Genomics Chromium**

Bomans, K. *et al.* (2018) Sepsis induces a long-lasting state of trained immunity in bone marrow monocytes. *Front Immunol.* 9:2685. doi:10.3389/fimmu.2018.02685.

*"Naïve bone marrow monocytes were isolated by negative selection using magnetic bead-based MACS Technology (Monocyte Isolation Kit (BM), Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Briefly, bone marrow cells were incubated with FcR Blocking Reagent and Monocyte Biotin-Antibody Cocktail. After washing, cells were incubated with Anti-Biotin MicroBeads and separation was facilitated on an autoMACS Pro Separator (Miltenyi Biotec, Bergisch Gladbach, Germany) via depletion of labeled non-monocyte cells"*

**Keywords: Mouse bone marrow, monocyte cells, untouched selection, autoMACS Pro Separator, Illumina HiSeq**

Tuttle, K. D. *et al.* (2018) TCR signal strength controls thymic differentiation of iNKT cell subsets. *Nat Commun.* 9.1:2650. doi:10.1038/s41467-018-05026-6.

*"Total thymocytes from BALB/c and SKG mice were incubated with PE-labeled anti-CD127 (A7R34), antiCD3 (145-201), anti-CD25 (PC61.5), anti-CD69 (H1.2F3), and PBS57/CD1d tetramers for 20 min at room temperature, washed and depleted using anti-PE magnetic beads and an autoMACS Pro Separator (Miltenyi Biotec)."*

**Keywords: Mouse thymus, thymocyte cells, anti-PE, anti-APC, positive selection, autoMACS Pro Separator, Illumina HiSeq 2500**



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