Miltenyi Biotec

Application note

Gentle sorting of PBMC populations

Background

Successful cell sorting via flow cytometry highly depends on the collection of viable cells. It is well established that high pressure, decompression, and shear forces negatively affect cell viability and functionality during conventional droplet sorting. Therefore, to mitigate the risk of cell damage, instrument settings, including nozzle size and pressure, have to be carefully chosen by experienced operators, who are capable of adjusting the settings accordingly. Sterility is another concern when working with traditional cell sorters that often operate in an uncontrolled, open-air surrounding. Samples are prone to contamination from both internal sources (sheath fluid, sampling fluidics, etc.) and external sources (airborne contaminants). Decontamination protocols using sterilizing agents such as bleach are long and tedious tasks that can affect the lasting viability of sorted cells. The use of antimicrobial media options in sort collection vessels, i.e. gentamicin and penicillin-streptomycin cocktails, can also hamper viability[1], affect cellular functions[2,3], or delay the recovery of cells sorted using traditional cell sorters. In addition, aerosol formation presents a hazard when sorting high-risk material, requiring precautions to ensure operator safety[4].

The MACSQuant® Tyto® is a benchtop cell sorter that is easy to use and fully closed, eliminating the above-mentioned concerns. At the heart of the system is the disposable, single-use cartridge which allows for completely aseptic sorting conditions with no chance of cross-contamination between samples. As sorting happens exclusively within the cartridge, there are no sheath fluid and fluidic tubings inside the instrument itself. Unlike on conventional droplet sorters, cells are sorted by a high-speed mechanical valve in a constant liquid stream of very low pressure within the MACSQuant Tyto Cartridge (fig. 1). Therefore, cells do not experience shear forces, they do not get decompressed or charged, and no potentially hazardous droplets form.
In this application note, we demonstrate the gentle sorting mechanism of the MACSQuant Tyto Sorter by sequentially enriching highly viable lymphocyte populations including B cells, T cells and natural killer (NK) cells from only one original sample of peripheral blood mononuclear cells (PBMCs).

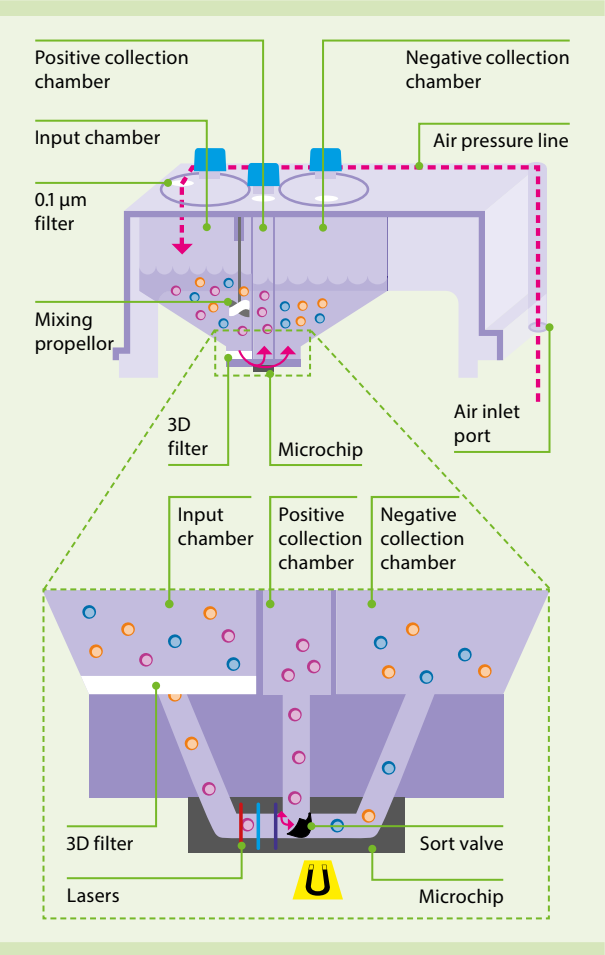


Figure 1: The sorting mechanism of the MACSQuant Tyto. Coming from the input chamber, cells enter the microchip through a microchannel where they get detected by the three lasers. Before entering the microchannel, potential cell aggregates are held back by a filter system to allow for a smooth sorting process. When a target cell (green) is identified, a magnetic pulse coming from the solenoid opens the microvalve, which then redirects the target cell into the positive collection chamber. In the default state, the valve is closed allowing non-selected cells (blue and red) to flow through into the negative collection chamber.

In this scenario, the negative non-sorted fraction from one sort becomes the input fraction for subsequent sorts (fig. 2). A new MACSQuant Tyto Cartridge is used during each sequential sort, preventing contamination from the previously sorted population and ensuring the sterility of all fractions. Most importantly, even after multiple sequential sorts, cell viability is not affected, demonstrating the gentle nature of the valve-mediated sorting mechanism.

Materials and methods

Staining

Following accepted practices for sterile technique, freshly obtained PBMCs of approximately 2×10^6 cells per mL were labeled with the 7-Color Immunophenotyping Kit. This kit includes a cocktail of fluorescently labeled antibodies targeting the human cell surface markers CD45, CD14, CD19, CD56/16, CD4, CD3 and CD8. Additionally, samples were treated with the viability marker Viobility 405/520 Fixable Dye to assess both pre- and post-sort viability. Once labeled, samples were transferred into a MACSQuant Tyto Cartridge and loaded into the MACSQuant Tyto Sorter. All materials supplied by Miltenyi Biotec.

Sort setup

Logical gating hierarchies were constructed using the MACSQuantify™ Software before sorting was initiated. Detailed, easy-to-follow protocols for both the labeling of PBMCs with the 7-Color Immunophenotyping Kit and other sorting strategies on the MACSQuant Tyto can be found in the appropriate data sheets.

Sort strategy

Cells were sorted in three passes (fig. 2) with each sequential sort targeting a unique population of lymphocytes: population 1 = $CD19^+$ B cells, population 2 = $CD8^+$ T cells, population 3 = $CD3^-/CD14^-/CD56^+/CD16^+$ NK cells. Freshly isolated PBMCs were the starting material for the initial B cell sort. The unsorted fraction was used as starting material for the subsequent T cell sort. Finally, the unsorted fraction from the T cell sort became the starting material for the NK cell sort.

Each subsequent sort was prepared using accepted practices for sterile technique and completed in a new MACSQuant Tyto Cartridge to ensure optimal sterility throughout the sorting cycle. Sorts were allowed to proceed for 60 minutes. Upon completion of all sorts, a fraction of the pre-sorted cells and a fraction from each of the positive and negative selected populations were evaluated for viability, purity, sort efficiency, sort yield, and overall yield using the MACSQuant Analyzer 10 (Miltenyi Biotec). Viability is determined by the percentage of sorted cells lacking the viability marker.

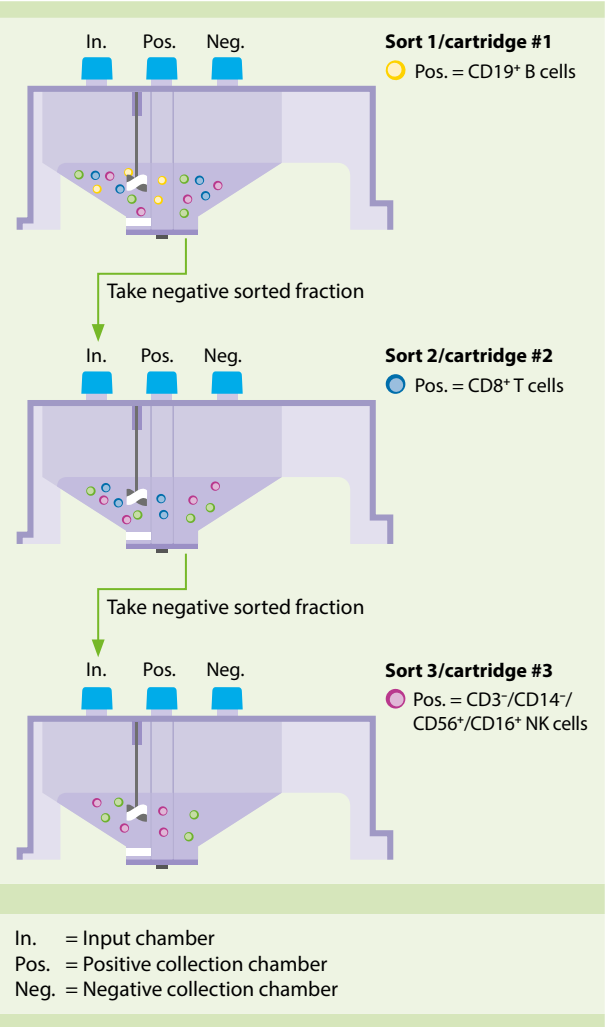
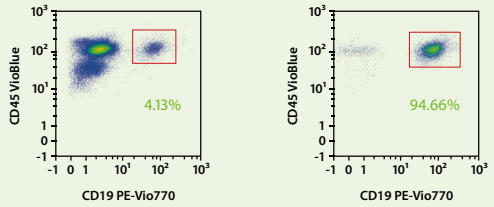


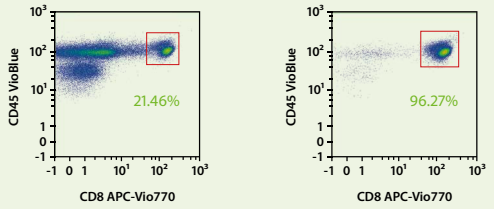
Figure 2: Sorting strategy for subsequent sorting of leukocyte populations on the MACSQuant Tyto Sorter.

1st sort: CD19+ B cells



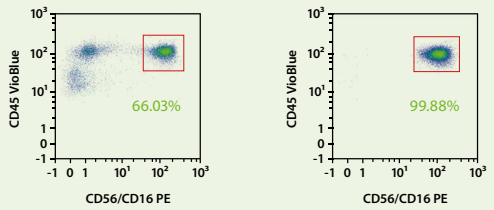
B cell sort outcome	
Purity	94.7%
Viability	98.9%
Sort efficiency	70.1%
Sort yield	73.0%
Overall yield	–

2nd sort: CD8+ T cells



T cell sort outcome	
Purity	96.3%
Viability	99.4%
Sort efficiency	66.9%
Sort yield	58.7%
Overall yield	50.8%

3rd sort: CD14-/CD3-CD56+/CD16+ NK cells



NK cell sort outcome	
Purity	99.0%
Viability	99.6%
Sort efficiency	63.6%
Sort yield	49.0%
Overall yield	29.2%

Results

Sort results demonstrate a selective enrichment of viable cell populations of interest and depletion of non-targeted populations for the CD19+ B cell, CD8+ T cell, and CD14-/CD3-/ CD56+/CD16+ NK cell sorts on the MACSQuant Tyto. In addition, pre- and post-sort plots are accompanied by a statistical evaluation, summarizing each sort outcome.

Purity represents the percentage of overall targeted cells in a sample versus all cells present in that sample. Sort efficiency is measured as a percent comparing the number of targeted cells found in the sorted fraction versus the number of targeted cells found in both the sorted and non-sorted fractions. Sort yield compares the number of targeted cells found in the sort fraction versus that present from the presorted fraction. Overall yield compares the number of targeted cells found in the sort fraction to the number of target cells in the original sample.

Conclusions

- Viability in each sort fraction is significant, approaching and exceeding 99% in each of the sequential sorts. This sorting strategy ensures the functionality of these sorted cells for downstream, cell-based assays.
- Sterility is maintained throughout the entire sorting process, enabling the integrity of downstream, cell-based applications and transplantation.
- Purity of the first sort exceeds 94% and improves with each subsequent sort reaching purities of up to 99%, providing material appropriate for sequencing, genomics, and gene editing-based applications.
- Ultimately, no sample is lost throughout the sorting workflow on the MACSQuant Tyto. The unsorted fraction from each sort is preserved in the negative sort chamber, providing the option to obtain additional target cells or seeking unique fractions from the same sample.

References

1. Fischer, A.B. (1975) Med. Microbiol. Immunol. 1: 23–39.
2. Relier, S. *et al.* (2016) Cancer Cell Int. doi: 10.1186/s12935-016-0277-6.
3. Ryu, A.H. *et al.* (2017) Sci. Rep. doi: 10.1038/s41598-017-07757-w.
4. Holmes, K.L. (2011) Cytometry A. 12:1000–1008.

Product table

Product	Order no.
MACSQuant Tyto Sorter	130-103-931
MACSQuant Tyto Cartridges	130-106-088
MACSQuant Tyto Running Buffer	130-107-207
7-color Immunophenotyping Kit, human	130-098-456
Viobility 405/520 Fixable Dye	130-109-814
MACSQuant Analyzer 10	130-096-343