Miltenyi Biotec

Gentle cell sorting, faster than ever: MACSQuantify™ Tyto® Software 3.2 redefines speed in microfluidic sorting

MACSQuant® Tyto Family of cell sorters

Evelyn Rodriguez-Mesa, Nadine Chelius, Daryl Grummitt, and Sudheer Gambheer

Background

The MACSQuant Tyto Family combines patented microchip technology with a closed cartridge system to deliver gentle and sterile cell sorting for advanced research, GMP-compliant workflows, and large-scale cell manufacturing.

In any sorting workflow, balancing purity and yield is critical, as overly stringent sorting can result in too few cells for meaningful analysis, while looser gating introduces unwanted cells that may skew the outcome. Although faster sorting can improve yield by processing more cells, exceeding the optimal event rate, typically 20,000–25,000 events/s in droplet sorters, may compromise purity and recovery. Traditionally, microfluidic sorters prioritize cell viability and sorting precision at the expense of yield and speed, a trade-off that once limited their use in high-throughput applications.

Here, we show that the MACSQuant Tyto Family redefines what's possible, combining gentle sorting technology with high-speed cartridges and powerful software automation. With the new MACSQuantify Tyto Software 3.2, microfluidic cell sorting is now faster than ever – up to 28 times faster than before – marking a new era of high-speed, high-purity, and high-viability sorting in a closed system.

Optimized efficiency with sort modes

The new MACSQuantify Tyto Software 3.2 features four automated sort modes (fig. 1), seamlessly optimizing the balance between purity and yield to specific experimental needs. Coincidence aborts, valve timing, and sort aborts are key parameters that are fine-tuned in each sort mode to achieve the desired outcomes (fig. 2).

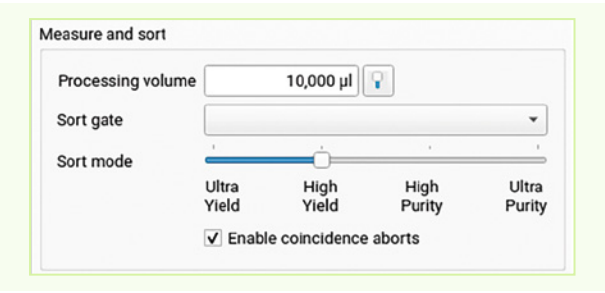

Figure 1: Sort mode options displayed in MACSQuantify Tyto Software 3.2.
The slider control displays the four sort modes, allowing users to easily adjust sorting preferences. The option to enable coincidence aborts is also available to enhance sorting accuracy.

Sort mode	Ultra Yield	High Yield	High Purity	Ultra Purity
Coincidence aborts	Disabled	Enabled	Enabled	Enabled
Sorting mechanism	Valve opens for all target cells.	Valve timing is adjusted to exclude nearby non-target cells. If exclusion fails, the target is still sorted.	Valve timing is adjusted to exclude nearby non-target cells. If exclusion fails, sorting is aborted.	Sorting occurs only if non-target cells are not detected nearby.
Valve opening time adjustment				

Target cell Non-target cell

Figure 2: Sorting mechanism for each sort mode. Coincidence aborts and valve opening time are fine-tuned in each sort mode to shift the balance between yield and purity. Ultra Yield mode maximizes recovery by disabling coincidence aborts and collecting all target cells, regardless of nearby non-targets. High Yield and High Purity modes balance purity and yield by enabling coincidence aborts and adjusting valve timing to exclude the nearby non-target cells, favoring yield in the former and purity in the latter. Ultra Purity applies the strictest criteria, ensuring maximal purity at the cost of lower yield.

In this study, we sought to explore how optimizing sort settings using the newly introduced sort modes in the MACSQuantify Tyto Software 3.2 influences the sorting performance of different cell types. Aiming to address the challenge of balancing purity and yield with speed, we used the MACSQuant Tyto Cartridge HS (high-speed), designed with inertial cell focusing technology for high-speed microfluidic cell sorting.

Methods

Cell culture and staining

These experiments were conducted using both delicate induced pluripotent stem cells (iPSCs) and robust peripheral blood mononuclear cells (PBMCs).

Human iPSC K10 cell line was cultured on iMatrix-511-coated 6-well plates using supplemented StemMACS™ PSC-Brew XF medium under standard conditions (37 °C, 5% CO_2). Cells were harvested and the total cell count was determined using the MACSQuant 10. 20% of the cells were stained with TRA-1-60-PE in MACSQuant Tyto Running Buffer, while the remaining 80% were blocked with TRA-1-60 pure antibody. Stained and unstained cells were kept separately at 4 °C until sorting. Before each sort, the two populations were mixed at an equal concentration of 1×10^6 cells/mL for the input sample.

Human PBMCs from healthy donors were stained with CD45-PE, CD3-VioBlue®, and CD4-APC. Cells were incubated at 4 °C for 10 minutes. Following staining, cells were washed once with PBS supplemented with 1% BSA, and then resuspended in MACSQuant Tyto Running Buffer at different concentrations: 1×10^6 cells/mL, 5×10^6 cells/mL, and 1×10^7 cells/mL.

Cartridge loading

The MACSQuant Tyto Cartridge HS was used for all sorts. Pre-sorting filtration of the samples was performed using a 10 mL syringe and a Pre-Separation Filter (20 µm). Cartridge priming and sample loading were executed following the MACSQuant Tyto Cartridge instructions. For each sort, the primed and sample-loaded cartridge was placed in the temperature-controlled chamber of the MACSQuant Tyto Cell Sorter set at 4 °C.

Cell sorting and analysis of the target cells

Cell sorting was carried out using the MACSQuant Tyto Cell Sorter and the brand-new MACSQuantify Tyto Software 3.2.

Viable iPSCs were gated based on TRA-1-60 expression (fig. 3) and sorted in all four modes. Viable $CD45^+CD3^+CD4^+$ T cells were sorted in the different concentrations (1×10^6, 5×10^6, and 1×10^7 cells/mL) using all four sort modes.

Flow cytometry analysis was performed on the MACSQuant Analyzer 10 using the MACSQuantify Software.

Calculated sort metrics

To evaluate the sorting performance using the sort modes, purity and sort efficiency were assessed by flow cytometry analysis applying the equations below. Sort efficiency was used as an indicator of yield, directly measuring the proportion of target cells recovered during sorting.

$$\text{Purity} = \frac{\text{number target cells pos. fraction}}{\text{total number cells pos. fraction}} \times 100\%$$

$$\text{Sort efficiency} = \frac{\text{number target cells pos. fraction}}{(\text{number target cells pos. fraction} + \text{number target cells neg. fraction})} \times 100\%$$

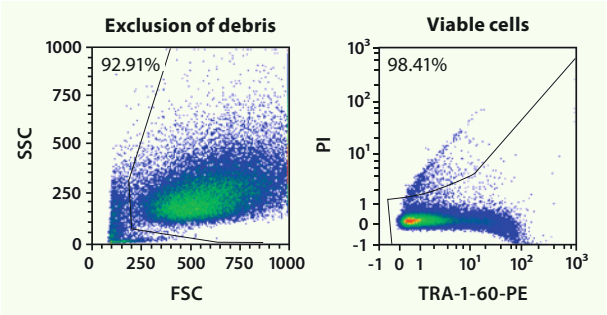

Figure 3: Gating strategy for the sorting of iPSCs. The sort gate was defined by excluding debris using scatter parameters and selecting viable TRA-1-60⁺ cells while excluding propidium iodide (PI⁺ dead cells).

Results

1. Sorting of delicate iPSCs using advanced sort modes

Stem cells, in particular iPSCs, are highly valuable for regenerative medicine and research but pose unique challenges in cell sorting due to their fragile nature and sensitivity to mechanical stress. Gentle sorting techniques are essential to preserve their functionality and differentiation potential, leading to better outcomes in research and clinical applications. With the release of four distinct sort modes in the latest MACSQuantify Tyto Software 3.2, we sought to determine how each mode influences the purity of delicate iPSCs.

Using the Ultra Purity mode, iPSCs were enriched from a target cell frequency of 20.58% in the input sample to 99.42% purity in the positive fraction (fig. 4A). Comparative analysis across the different sort modes revealed that, while the Ultra Purity mode delivers the highest sample purity, the High Purity mode closely followed at 99.20%. In contrast, the yield-focused modes delivered lower purities, with the High Yield mode reaching 94.10% and the Ultra Yield mode achieving the lowest purity at 92.90% (fig. 4B).

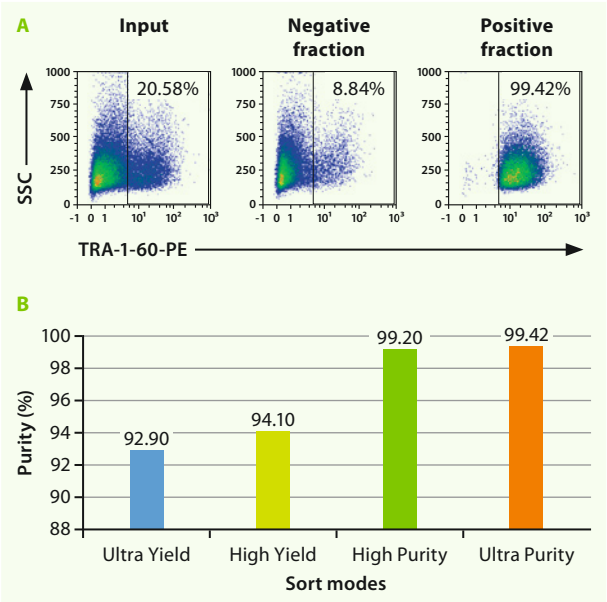

Figure 4: Cell sorting of TRA-1-60⁺ iPSCs using the different sort modes. All fractions (input, negative, and positive) were analysed in the MACSQuant 10. Cell sorting using the Ultra Purity mode enabled gentle isolation of iPSCs with purity above 99% (A). Sample purity of iPSCs improves from yield-focused to purity-focused sort modes (B).

These results show that the purity-focused modes (High Purity and Ultra Purity) are significantly more effective at enriching iPSCs to near 100% purity than the yield-focused modes. However, even the yield-focused modes enable high sample purities, above 92%, demonstrating the effectiveness of the MACSQuant Tyto Family of cell sorters in maintaining substantial purity while maximizing cell recovery.

2. Purity-focused sort modes enable high-speed performance without compromising sample purity

To evaluate how the different sort modes balance purity, yield, and instrument speed, CD45⁺CD3⁺CD4⁺ T cells were sorted at increasing concentrations, from 1×10^6 to 5×10^6 cells/mL and 1×10^7 cells/mL. Sample purity (fig. 5A) and sort efficiency (fig. 5B) were assessed and plotted against the event rates, a key metric of instrument speed. Sort efficiency was used to measure yield, indicating how effectively target cells were recovered during sorting.

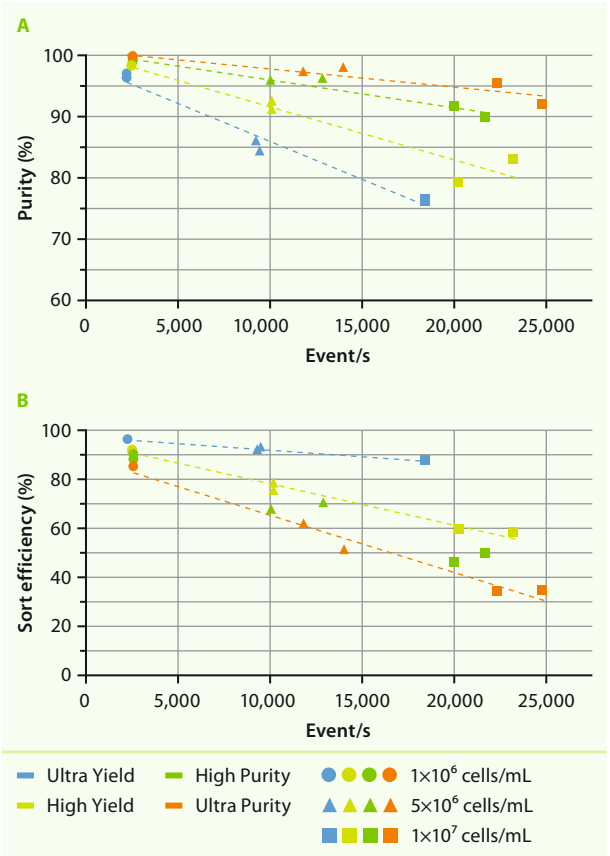

Figure 5: Evaluation of sort metrics using the sort modes in the MACSQuantify Tyto Software 3.2. T cells were sorted in different concentrations using the four sort modes. Purity (A) and sort efficiency (B) were plotted against event rate, a key metric for the instrument speed. Sort efficiency was used as a measure of yield.

At low sample concentration (1×10^6 cells/mL), all sort modes achieved purities above 96% (fig. 5A), confirming the ability of the MACSQuant Tyto Family to consistently deliver high purity across modes, similar to the results obtained with iPSCs.

The data reflect the inherent trade-off between purity and yield across the sort modes. The Ultra Purity mode maintained the highest purities, even at higher event rates, at the expense of yield. In contrast, the Ultra Yield mode preserved the highest sorting efficiencies with a reduction in sample purity.

At higher sample concentrations (5×10^6 cells/mL), the Ultra Yield mode continued to deliver the highest sort efficiencies (above 90%) (fig. 5B), whereas the High Yield mode offered a favorable compromise, sustaining efficiencies above 75% with improved purities exceeding 90% (fig. 5A, B).

Strikingly, at a sample concentration of 5×10^6 cells/mL, the Ultra Purity mode achieved sample purities exceeding 97% at 12,000–14,000 events/s (fig. 5A), a 28-fold speed increase over the previous software versions.
This result shows that the purity-focused sort modes offer high-speed performance while prioritizing purity at the expense of yield (fig. 5B). Similarly, at a sample concentration of 1×10^7 cells/mL, the High Purity and Ultra Purity modes deliver sample purities above 90% at event rates ranging from 22,000 to 25,000 events/s (fig. 5A) – a groundbreaking speed for microfluidic sorting, previously thought achievable only with traditional droplet-based systems.

Together, these results show that the new sort modes in the MACSQuantify Tyto Software enable high-purity performance at impressive speeds, up to 28 times faster than before. In combination with the gentle sorting technology, the MACSQuant Tyto Family achieves droplet sorter-level speed without compromising on cell viability and purity.

Conclusions

Achieving a high event rate must be carefully managed to maintain an optimal balance between purity and yield, ensuring robust performance. The MACSQuantify Tyto Software 3.2 introduces four sort modes delivering tailored sorting performance to meet diverse experimental needs.

Ultra modes for targeted results
The Ultra Yield and Ultra Purity modes offer peak performance at opposite ends of the sorting spectrum – maximizing either cell recovery or purity, respectively. The Ultra Purity mode is ideal for applications where maximal purity is critical, such as cell manufacturing workflows or sensitive downstream assays. On the other edge, the Ultra Yield mode excels when cell numbers are limited, making it ideal for pre-enrichment steps or low-input samples.

Flexible modes for real-world applications
The High Yield and High Purity modes provide a strategic middle ground, striking the right balance for workflows that require both reliable purity and high cell recovery, offering versatility for diverse experimental needs.

High-speed performance meets high purity
The Ultra Purity and High Purity modes maintain exceptional purity even at elevated event rates, making them the ideal solution for high-throughput applications where stringent purity standards are non-negotiable.

Breaking the speed barrier:
Microfluidics meets droplet-level performance
Droplet sorters have long been the gold standard for sorting speed, operating at 20,000–25,000 events/s with optimal performance, a range once out of reach for microfluidic systems. With the introduction of sort modes, the MACSQuant Tyto Family redefines that benchmark, achieving droplet sorter-level speed while preserving the benefits of a gentle and closed sorting system.

Developed for precision and flexibility, the new sort modes integrated in the MACSQuantify Tyto Software 3.2 empower users to optimize sorting performance based on their specific requirements for yield or purity. When paired with the MACSQuant Tyto Cartridge HS, the system propels microfluidic cell sorting into high-speed applications, delivering faster sorting than ever before. With industry-leading safety and gentle sorting technology, the MACSQuant Tyto Family now offers a unique blend of speed, purity, and cell viability that traditional droplet-based systems simply can't match.

Product	Order no.
MACSQuant Tyto Cell Sorter	130-103-931
MACSQuant Tyto Cartridge HS, 24 pieces	130-121-551
MACSQuant Tyto Calibration Beads	130-122-730
MACSQuant Tyto Running Buffer	130-107-206
Pre-Separation Filter (20 µm)	130-101-812
StemMACS PSC-Brew XF, human medium	130-127-865
CD45-PE, anti-human, REAfinity®	130-110-632
CD3-VioBlue, anti-human, REAfinity	130-114-519
CD4-APC, anti-human, REAfinity	130-113-222
TRA-1-60-PE, anti-human, REAfinity	130-122-921
Propidium Iodide Solution	130-093-233

VISIT

Master even the most advanced sorting experiments with MACSQuantify Tyto Software.
▸ **miltenyibiotec.com/tyto-software**

LEARN MORE

Explore the MACSQuant Tyto Family and unlock new possibilities in cell sorting today!
▸ **miltenyibiotec.com/tytofamily**

Miltenyi Biotec

Miltenyi Biotec B.V. & Co. KG | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macsde@miltenyi.com | **www.miltenyibiotec.com**
Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

130-139-166