Miltenyi Biotec

Gentle and safe sorting of cells expressing fruit dyes

Meet the new MACSQuant® Tyto® Lux Cell Sorter

Evelyn Rodriguez-Mesa, Daryl Grummit, and Alina Steinbach

Background

Cell line development is widely used in research, diagnostics, and biopharmaceutical production, enabling the development of therapeutic proteins, monoclonal antibodies, and vaccines. However, challenges like selecting optimal clones, ensuring genetic stability, and scaling up for consistent manufacturing can complicate the process. Gentle and sterile cell sorting is key to overcoming these challenges by enabling the isolation of high-performing, stable clones while preserving cell integrity and supporting reliable, efficient cell line development.

The MACSQuant Tyto Lux Cell Sorter

In contrast with conventional droplet sorters, the unique microchip-based sorting technology of the MACSQuant Tyto Family mitigates sorter-induced cellular stress, preserving cell viability and functionality. The closed MACSQuant Tyto Cartridge keeps samples sealed and sterile throughout the cell sorting, eliminating the contamination risk for both the sample and the operator (fig. 1).

The MACSQuant Tyto Lux Cell Sorter adds a 552 nm yellow laser to the established violet, blue, and red channels, enhancing sorting flexibility. The integrated MACSQuantify™ Tyto Software 3.2 offers next-level automation, including four automated sorting modes (ultra-yield, yield, purity, ultra-purity) and three laser modes (Yellow Only, Yellow Off, Yellow/Blue Balanced) for maximum versatility and efficiency.

Exploring the flexibility and automation provided by the MACSQuant Tyto Lux Cell Sorter, this study sought to sort HEK293 cells expressing the fruit dye mCherry. For posterior analysis, the MACSQuant VYB Analyzer was chosen, building a powerful team with the MACSQuant Tyto Lux Cell Sorter.

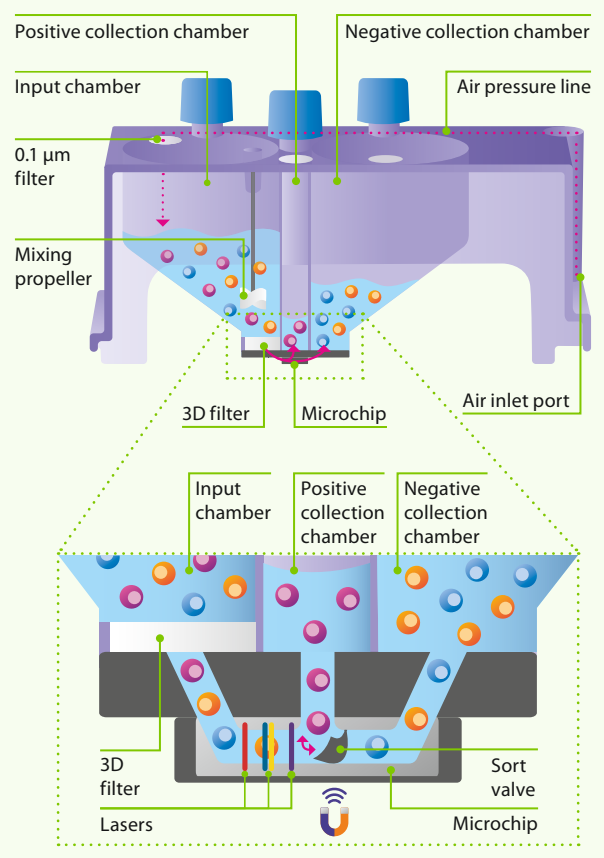

Figure 1: The sorting mechanism of the new MACSQuant Tyto Lux Cell Sorter. Upon inserting the cartridge into the instrument, air from the instrument is directed into the input chamber through a 0.1 µm filter. The air flow propels the cells through a 3D filter system before guiding them into the microchannel and toward the microchip. Inside the microchip, cells are interrogated by laser beams. Fluorescent and scattered light signatures are used to identify cells. Non-target cells (orange, blue) bypass the closed valve into the negative collection chamber. Once a target cell is identified (pink), a magnetic pulse opens the sorting valve, redirecting the target cell into the positive collection chamber.

Methods

Cell culture

The widely used human embryonic kidney cell line HEK293 was chosen as a model system to express the reporter protein, mCherry.

HEK293 expressing mCherry and wild-type K562 cells were cultured on T25 flasks in RPMI medium supplemented with 10% FBS and 1% penicillin/streptavidin, under standard conditions (37 °C, 5% CO_2).

Staining and cartridge preparation

On day 4 of culture, cells were harvested from the flasks using incubation with trypsin for 5 minutes, and the cell count was determined using the MACSQuant VYB Analyzer. Cells were then stained with CellTrace™ CFSE (Thermo Fisher Scientific) according to the manufacturer's instructions. Following staining, the mCherry-expressing cells were mixed with wild-type K562 cells and resuspended in Tyto Running Buffer at a concentration of 1×10^6 cells/mL. Staining with DAPI Staining Solution was performed to exclude dead cells during subsequent analysis.

Pre-filtration of the samples before sorting 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. The primed and loaded cartridge was placed in the temperature-controlled chamber of the MACSQuant Tyto Lux Cell Sorter set at 4 °C.

Cell sorting and analysis of the target cells

Cell sorting was carried out using the MACSQuant Tyto Lux Cell Sorter and the MACSQuant Tyto Cartridge HS (high-speed). Ultra-purity sorting mode and Yellow Only laser mode were selected for cell sorting.

Gating strategy

After excluding debris and gating on live cells (DAPI-negative), the sort gate was defined on HEK293 cells expressing both CellTrace-CFSE (measured in B1 channel) and mCherry (measured in B + G3 channel) (fig. 2).

After sorting, analytical flow cytometry was performed on the MACSQuant VYB Analyzer. Cells were in addition visually assessed for their shape and fluorescent expression, using the Olympus IX70 fluorescence microscope.

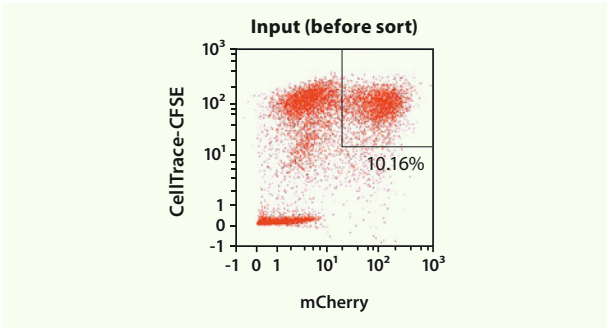

Figure 2: Gating strategy for cell sorting on the MACSQuant Tyto Lux Cell Sorter. After debris exclusion by scatter channels, the sort gate was defined on live cells (negative for DAPI staining) that are positive for both CSFE and mCherry. Cells were sorted in Yellow Only laser mode.

Results

The MACSQuant Tyto Lux Cell Sorter enables the successful isolation of highly viable and pure mCherry-transduced cells

After sorting CellTrace-CFSE and mCherry double-positive HEK293 cells on the MACSQuant Tyto Lux Cell Sorter, cell fractions were recovered from the input, positive, and negative cartridge chambers and individually analyzed by flow cytometry. While the target cell population constituted 11.65% of the total viable cells in the input fraction and 5.02% of the negative fraction, CellTrace-CFSE and mCherry double-positive HEK293 cells were enriched to a purity of 99.10% in the positive fraction (fig. 3). Sorted cells displayed 98.85% cell viability, higher than the input (94%), showing effective depletion of dead cells (fig. 3 and table 1). As the ultra-purity mode was selected, sorting is optimized to achieve high purity (99.10%), at the expense of higher yield (51.97%) (table 1).

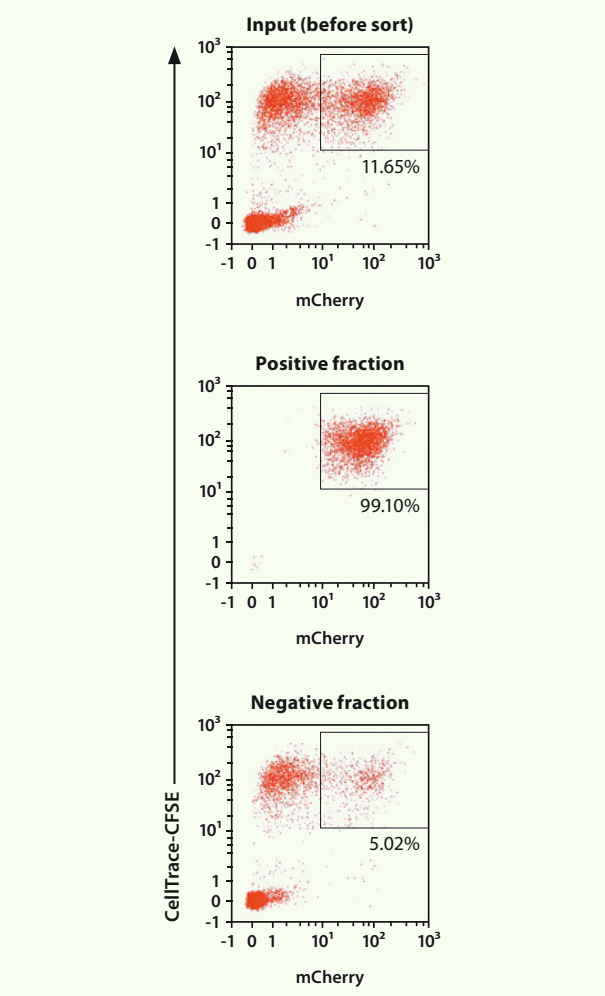

Figure 3: Flow cytometry analysis of transduced cells after cell sorting on the MACSQuant Tyto Lux Cell Sorter. Cells expressing both CellTrace-CFSE and mCherrry were selected as the target population and sorted in ultra-purity mode. Flow cytometry analysis of the input, positive, and negative fractions was posteriorly performed on the MACSQuant VYB Analyzer. Dot plots show all cells before sorting (input), CellTrace-CFSE and mCherrry double-positive target cells after sorting (positive fraction), and the cell fraction depleted of target cells (negative fraction).

Summary of sort parameters	
Purity*	99.10%
Yield	51.97%
Viability before sort (input)	94%
Viability after sort (positive fraction)	98.85%

* Cells were sorted in ultra-purity mode

Table 1: Summary of the sort metrics is displayed in percentages. Ultra-purity mode favors high purity of the target cells at the expense of greater yield.

Cells from the input and positive fractions were in addition visually assessed for their shape and fluorescent expression under the microscope. Overlay of the brightfield and fluorescent channels shows strong enrichment for double-positive cells in the positive fraction, in comparison to the input fraction (fig. 4). Importantly, the rounded cell morphology in the positive fraction indicates that cells are healthy and intact after cell sorting.

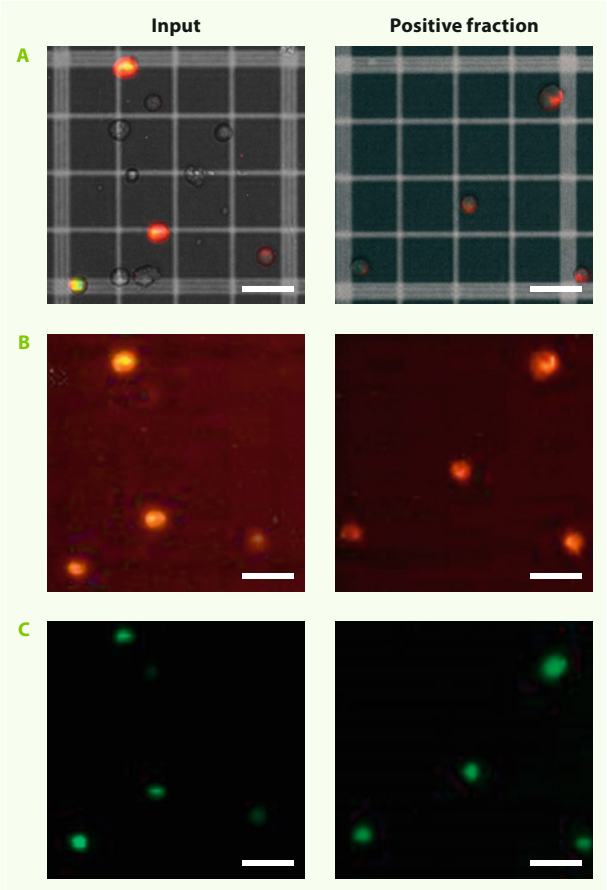

Figure 4: Microscopy analysis of sorted cells. Cells from the input and positive fractions were assessed under the microscope. Overlay of fluorescent and brightfield channels (a), mCherry (b), and CellTrace-CFSE (c) are depicted. Scale bar: 50µm.

Conclusions

The MACSQuant Tyto Lux Cell Sorter offers all the essential benefits for efficient, reliable sorting of cells expressing fruit dyes:

- **Enhanced flexibility:** The additional yellow laser (552 nm) enables precise sorting of transduced cells expressing reporter genes like mCherry, expanding experimental capabilities.
- **Advanced software automation:** Standardization features, such as the automated sorting modes, maximize performance, boosting efficiency, reproducibility, and scalability.
- **Gentle sorting:** The microchip-based cell sorting preserves cell viability and functionality, which is crucial for maintaining high-performing, stable clones.
- **High efficiency:** The ultra-purity sort mode delivers highly concentrated and pure cell populations, ensuring efficient clone selection, fast cell expansion, and minimized risk of contamination.
- **Sterile & safe:** The closed cartridge keeps cells sealed and sterile, preserving sample integrity and minimizing the risk of culture failure.

Product	Order no.
MACSQuant Tyto Lux Cell Sorter	130-133-903
MACSQuant Tyto Cartridge HS, 8 pieces	130-121-549
MACSQuant Tyto Cartridge, 24 pieces	130-121-551
MACSQuant Tyto Calibration Beads	130-122-730
MACSQuant Tyto Running Buffer	130-107-206
MACSQuant VYB Analyzer	130-096-116
DAPI Staining Solution	130-111-570
Pre-Separation Filter (20 µm)	130-101-812

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Miltenyi Biotec B.V. & Co. KG | Friedrich-Ebert-Straße 68 | 51429 Bergisch Gladbach | Germany | Phone +49 2204 8306-0 | Fax +49 2204 85197
macsde@miltenyi.com | **www.miltenyibiotec.com**

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