Miltenyi Biotec

Sorting of large cells: Primary hepatocytes

MACSQuant® Tyto® Family of cell sorters

Important note: This AppNote is only applicable when using the MACSQuant Tyto Cartridge LC and MACSQuantify™ Tyto Software 3.2

Background

In the extensive landscape of liver research and related therapeutic applications, hepatocytes emerge as pivotal cell entities, indispensable for deciphering intricate liver functions and conducting essential toxicity studies[1]. However, sorting primary hepatocytes can be challenging. Their large size, complex cellular structure, and high metabolic activity make them extremely sensitive to sorter-induced cellular stresses (SICS). As a consequence, cell sorting can result in cellular damage and poor cell viability[2], compromising downstream applications.

In contrast to conventional droplet sorters, the MACSQuant Tyto Family of benchtop cell sorters uses a high-speed mechanical valve to sort cells within a low-pressure liquid stream (fig. 1). This gentle microchip-based sorting technology eliminates shear forces, decompression, electrical charge, and high pressures, mitigating SICS and preserving cell functionality. Cell sorting occurs in a sterile, disposable cartridge, preventing contamination and sample carry-over.

In this application note, we provide a complete workflow for gentle cell sorting of primary mouse hepatocytes – from tissue perfusion and cell preparation using gentleMACS™ Perfusion Technology, to subsequent microchip-based cell sorting with MACSQuant Tyto Cell Sorter. Cell sorting was performed using the new MACSQuant Tyto Cartridge LC (large-cell), specially designed to sort large cells with diameters ranging from 20 to 50 µm.

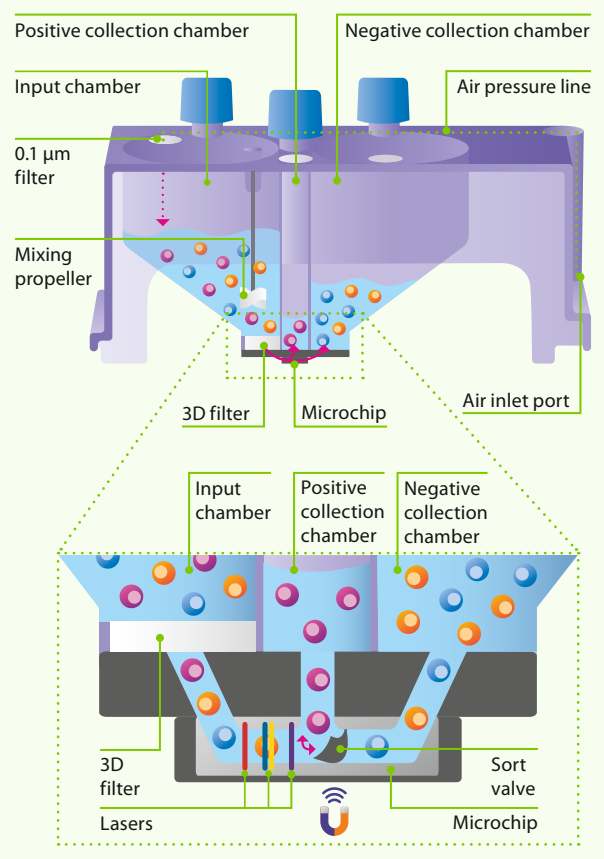

Figure 1: The sorting mechanism of the MACSQuant Tyto Cartridge. Upon inserting the cartridge into the instrument, air from the instrument is directed into the input chamber through a 0.1 µm filter. The air flow propels the cells through a 3D filter system before guiding them into the microchannel and 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. The valve then returns to its original position and is ready for the next sort.

Methods

Tissue perfusion and hepatocyte preparation
Isolation of viable primary hepatocytes from liver tissue was performed using the Liver Perfusion Kit and gentleMACS Perfusers, on the gentleMACS Octo Dissociator with Heaters. For details, please refer to the Miltenyi Biotec liver perfusion video protocol (see page 3).

Staining and cartridge preparation
Approximately 5×10^6 total viable hepatocytes were stained with APC-conjugated REAfinity® CD95 (FAS) Antibody, at a dilution of 1:50 in MACSQuant Tyto Running Buffer, following Miltenyi Biotec cell surface flow cytometry staining protocol (see page 3). Cells were centrifuged at 30×g for 5 minutes and washed once with MACSQuant Tyto Running Buffer. Pre-filtration of the sample was performed using Pre-Separation Filters (70 µm), before loading it into the MACSQuant Tyto Cartridge LC. Cartridge priming and sample loading were performed following the MACSQuant Tyto Cartridge instructions.

Cell sorting and analysis of target cells
Cell sorting was carried out using the MACSQuant Tyto Cell Sorter and MACSQuantify Tyto Software version 3.2.
The input sample contained 5×10^5 total viable cells/mL in MACSQuant Tyto Running Buffer. The gating strategy was to select CD95-APC and autofluorescent FITC double-positive hepatocytes.

Flow cytometry analysis was performed on the MACSQuant Analyzer 10 using the MACSQuantify Software. Expression of lineage markers was assessed and cell purity, yield, and cell viability were quantified.

Cell culture after sorting
To assess the impact of the cell sorting process on the hepatocytes, cells were recovered from the positive chamber and incubated on collagen-coated 48-well plates in hepatocyte cultivation medium (HepatoZYME-SFM, Thermo Fisher Scientific, supplemented with 5% FBS, 1% penicillin/streptavidin, and 1% L-glutamine), at 37 °C and 5% CO_2.

Results

The gentle sorting provided by MACSQuant Tyto in combination with the MACSQuant Tyto Cartridge LC, enabled successful isolation of viable primary hepatocytes
The MACSQuant Tyto enriched the target antigen-specific $CD95^+$ cells from 63.9% to 96.2% (fig. 2). A total of 3.72×10^6 cells were recovered from the positive collection chamber, representing 85% yield of target cells from the total input fraction. Cell viability was preserved during the sorting process, remaining similar to the input fraction, above 77%, in the sorted cells.

The high percentage of target cells in the negative collection chamber, 22.6%, is attributed to the high frequency of target cells in the input fraction and overall low number of non-target cells.

Sorted hepatocytes displayed robust replating ability and typical morphological features in culture
After 24 hours of cultivation, the sorted hepatocytes were attached and exhibited typical hexagonal morphology and nuclei duplication (fig. 3), indicating healthy cells and normal cellular function.

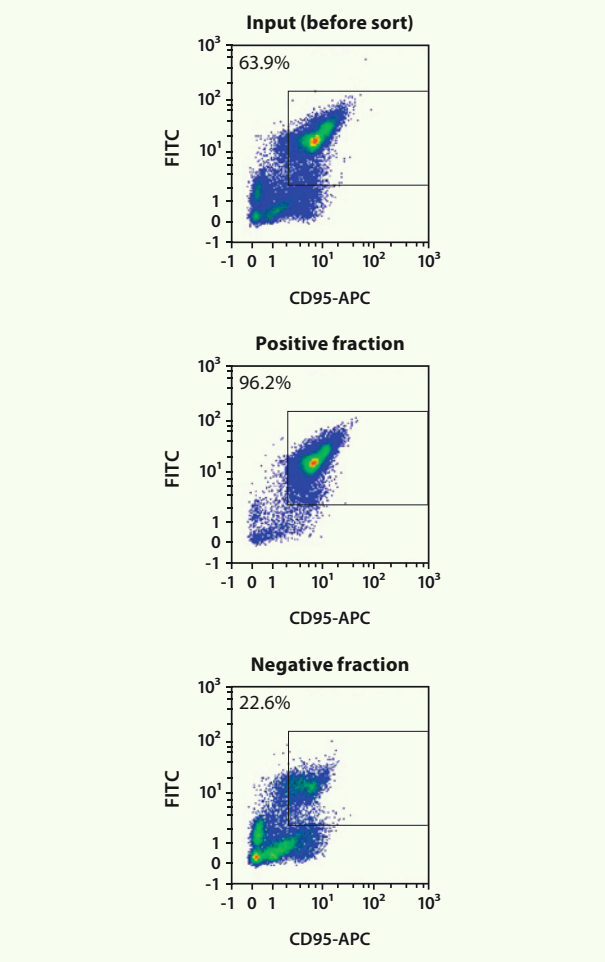

Figure 2: Sorting of $CD95^+$ hepatocytes with the MACSQuant Tyto and the MACSQuant Tyto Cartridge LC. Primary hepatocytes from mice were labeled with CD95-APC and sorted on the MACSQuant Tyto. Flow cytometry analysis was performed on the MACSQuant Analyzer 10. Dot plots show all cells before sorting (input), the CD95-APC target cell fraction after sorting (positive fraction), and the cell fraction depleted of CD95-APC cells (negative fraction).

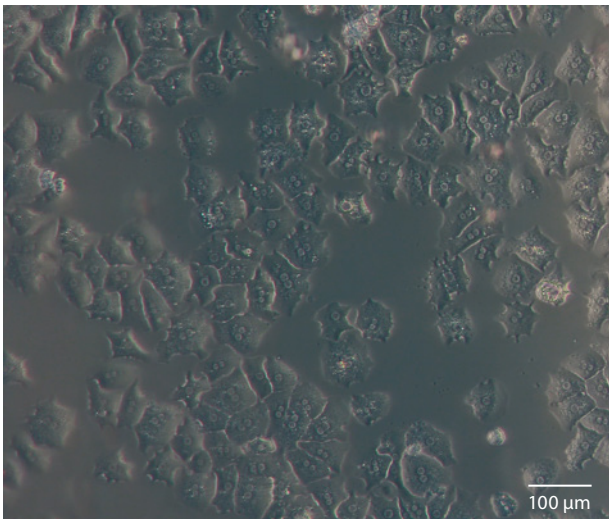

Figure 3: Cell morphology of sorted $CD95^+$ cells, after 24 hours in culture. Brightfield imaging of hepatocytes recovered from the positive collection chamber showed cell attachment, hexagonal cell shape, and nuclei duplication.

Conclusions

Here, we present a comprehensive workflow for mouse hepatocyte isolation, starting with liver perfusion using the gentleMACS Perfusion Technology, followed by cell sorting using the MACSQuant Tyto Cartridge LC on the MACSQuant Tyto Cell Sorter.

This workflow enabled the successful cell sorting of large cells with a diameter up to 31 µm. Primary mouse hepatocytes were isolated with a purity exceeding 96%, while maintaining the pre-sort viability superior to 77% in the sorted fraction. The isolated hepatocytes showed robust post-sorting plating capacity and exhibited typical morphological features, including hexagonal shape and nuclei duplication. These results indicate that primary hepatocytes were effectively sorted, with preservation of their viability and phenotypic properties in culture. Further comprehensive assays are needed to fully assess the extent of preserved cell functionality.

In conclusion, the MACSQuant Tyto Family's advanced microchip-based gentle cell sorting technology, in conjunction with the MACSQuant Tyto Cartridge LC, sets a new standard for successfully sorting large, delicate cells such as primary hepatocytes.

References

1. Hughes, R. D. *et al.* (2006) J. R. Soc. Med. 98: 341–345.
2. Schulze, R. J. *et al.* (2019) J. Cell Biol. 218: 2096–2112.

Product	Order no.
MACSQuant Tyto Cell Sorter	130-103-931
MACSQuant Tyto Lux Cell Sorter	130-133-903
MACSQuant Tyto Cartridge LC	130-133-260
MACSQuant Tyto Running Buffer*	130-107-206
MACSQuant Analyzer 10	130-096-343
Liver Perfusion Kit, mouse and rat	130-128-030
gentleMACS Perfusers	130-128-151
gentleMACS Octo Dissociator with Heaters	130-096-427
CD95 (FAS) Antibody, anti-mouse, APC, REAfinity	130-123-568
Pre-Separation Filters (70 µm)	130-095-823

*For research use only

VIDEO

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GET A PROTOCOL

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LEARN MORE

Explore the MACSQuant Tyto Family of cell sorters and unlock new possibilities in cell sorting today!

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