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Advance your T cell research with a closed and sterile system

Isolation of human regulatory T cells (Tregs) with the CliniMACS Prodigy® and the MACSQuant® Tyto® Cell Sorter

Introduction

Regulatory T cells (Tregs) are a rare subpopulation of immune cells that circulate at 1–4% of peripheral blood mononuclear cells.[1] Although they comprise a small fraction of immune cells, Tregs play a powerful role in maintaining immune homeostasis by downregulating overactive immune responses.[2] Regulatory T cells are derived from the same lineage as $CD4^+$ cells and express the CD4, CD25, and FoxP3 biomarkers.[3] Due to their immunosuppressive activity, Tregs are being studied extensively as biological therapeutics for autoinflammatory diseases and for their ability to impart an immune tolerance to transplanted cells and organs.[4] With dozens of Treg clinical trials in progress, the need to produce these immune cells with high purity and viability is increasing.

The MACSQuant Tyto is an easy-to-use benchtop cell sorter that enables rapid isolation of Tregs. At the heart of the system is the disposable, single-use cartridge, which allows for completely aseptic sorting conditions with no risk of cross-contamination between samples. This closed-cartridge system provides protection for your samples and safety from aerosols or droplets (fig. 1). With low-pressure fluidics and no decompression, the gentle system supports high cell viability and re-sorting of functional cells.[5] The MACS® Cartridges, both standard and high-speed, are available on the MACSQuant Tyto. The high-speed cartridges deliver the same single-use aseptic sorting conditions and high-quality performance in half the sorting time. Here we demonstrate the capacity of the CliniMACS Prodigy and MACSQuant Tyto systems with the MACSQuant Tyto Cartridge HS (High-Speed) to sort rare antigen-specific Tregs from white blood cells. We also show successful cell expansion using the Miltenyi Biotec Treg Expansion Kit.

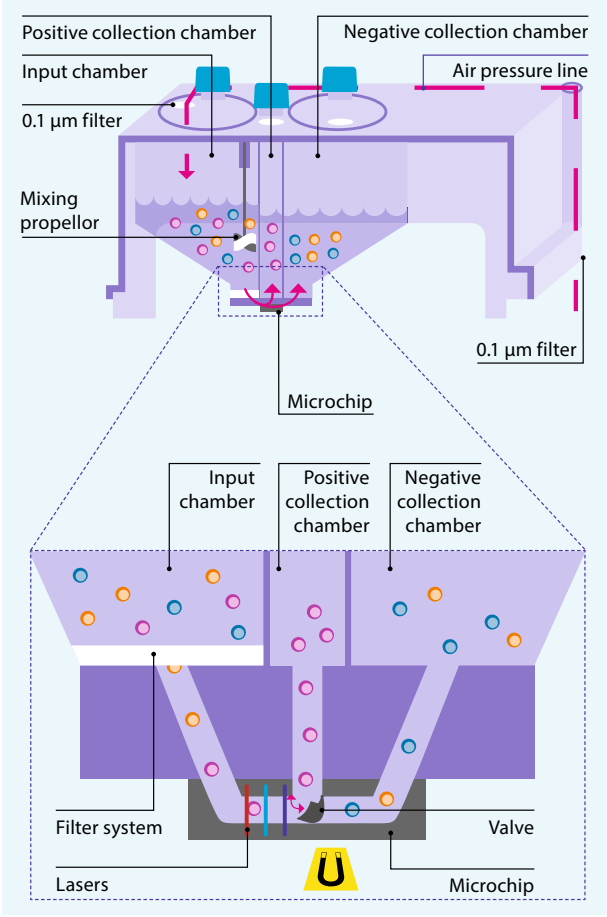


Figure 1: The sorting mechanism of the MACSQuant Tyto Cartridge. En route from the input chamber, cells enter the microchip through a microchannel where they are detected by three lasers. When a target cell (pink) is identified, a magnetic pulse opens the microvalve, which then redirects the target cell 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.

Methods

CD25⁺ pre-enrichment with the CliniMACS Prodigy

In this study, white blood cells obtained via leukapheresis were pre-enriched on the CliniMACS Prodigy. CliniMACS Prodigy LP-25 Pre-enrichment enables automated, magnetic pre-enrichment and fluorescent labeling of CD25⁺ cells that prepares them for sorting on the MACSQuant Tyto. A MACS Peripheral Blood ¼ Leukopak (Miltenyi Biotec, # 150-000-451) containing approximately 2.5×10^9 cells was loaded onto the CliniMACS Prodigy and parameters were set using the CliniMACS LP-25 program, according to the manufacturer's instructions.
As FoxP3 is an intracellular protein, and the CD127 extracellular marker is known to inversely correlate with FoxP3 expression, we used CD127$^{dim/-}$ expression as part of the Treg isolation strategy.[6] Cells were enriched with MACS GMP CD25-PE Biotin (Miltenyi Biotec, # 170-076-505), and then labeled with MACS GMP CD127-APC (Miltenyi Biotec, # 170-076-501), MACS GMP CD4-VioBlue (Miltenyi Biotec, # 170-076-504) and CD25-PE. The final product was eluted in 20 mL MACSQuant Tyto Running Buffer with Tytonase (130-107-206). The pre-enrichment run time took approximately 5 hours.

CD4⁺CD25⁺ CD127$^{dim/-}$ sorting with MACSQuant Tyto Cell Sorter

After enrichment, cells were sorted sequentially on the MACSQuant Tyto Cell Sorter based on the sorting criteria CD4⁺CD25⁺CD127$^{dim/-}$. To model a clinical workflow, the CD25⁺ enriched cells were divided into three high-speed cartridges for three sequential debulk sorting steps, followed by a purity sort using one high-speed cartridge. The input for each debulk sort contained 6.3×10^6 cells/mL in 10 mL MACSQuant Tyto Running Buffer and took 1.25 hours per run. The input for the purity sort contained 2.6×10^6 cells/mL in 4 mL buffer and took 0.5 hour to complete. The full MACSQuant Tyto process took 4.25 hours total (3 debulking runs and 1 purity run).
The total hands-on workflow time for the CliniMACS Prodigy pre-enrichment and MACSQuant Tyto sort was approximately 10 hours, divided between 2 days.

Cell expansion

As Tregs represent a small cell population, typically 1–2% of total lymphocytes, many downstream applications require an expansion of Tregs prior to analysis. Regulatory T cells sorted by the MACSQuant Tyto were expanded using the Treg Expansion Kit (Miltenyi Biotec, # 130-095-345). Cells were expanded in TexMACS™ Medium (Miltenyi Biotec, # 130-097-196) and supplemented with 500 U/mL recombinant IL-2 (Miltenyi Biotec, # 130-097-748) and 5% AB serum.
Cells were harvested at 14 days, counted and fluorescently stained with Anti-FoxP3-PE.



Figure 2: After enrichment on the CliniMACS Prodigy, cells were sorted sequentially on the MACSQuant Tyto Cell Sorter.

Results

Flow cytometry analysis was performed with MACSQuantify™ Software after completion of each of the following: CliniMACS Prodigy enrichment, MACSQuant Tyto debulk sort, and MACSQuant Tyto purity sort and cell expansion. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

To assess the purity of CD4⁺CD25⁺ Tregs, the CliniMACS Prodigy-enriched sample was first analyzed for expression of CD4 (y-axis) and CD25 (x-axis) (fig. 2) and then plotted to indicate CD25-PE (y-axis) vs CD127-APC (x-axis) expression utilizing the MACSQuant Analyzer 10. The data demonstrated that target cells were enriched to 38% (fig. 2).

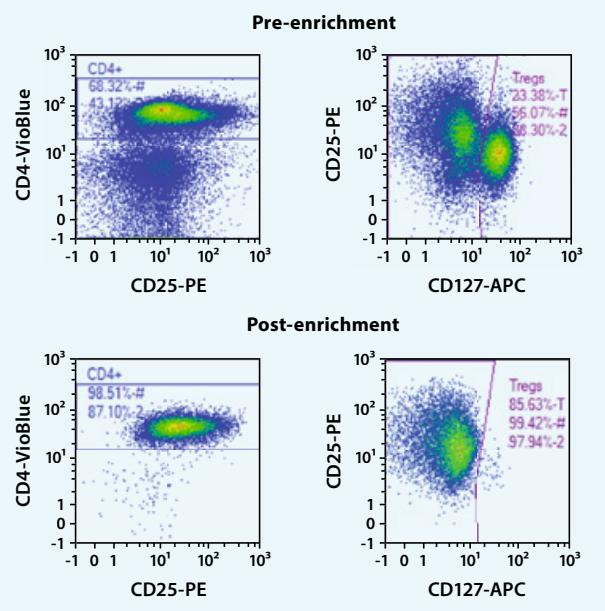


Figure 3: Purity of Tregs after pre-enrichment and cell sorting. After pre-enrichment on the CliniMACS Prodigy Tregs (CD4⁺CD25⁺CD127$^{dim/-}$) were enriched to 38%. With the help of the MACSQuant Tyto Cell Sorter the purity could be successfully increased to 98%.

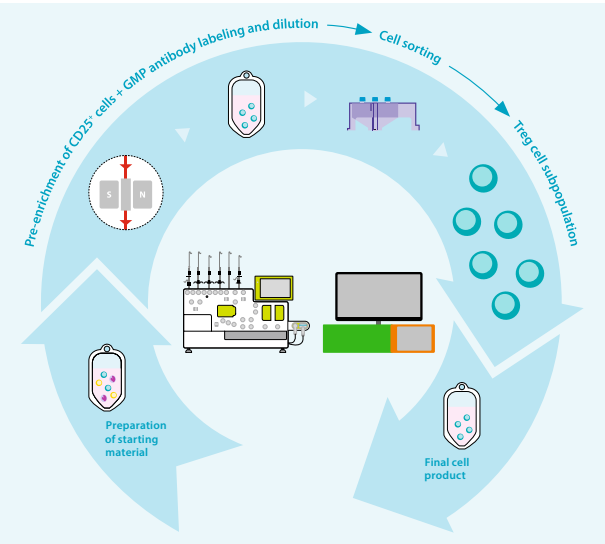


Figure 4: Overview of the regulatory T cell enrichment using the CliniMACS Prodigy and the MACS Quant Tyto Cell Sorter.

To assess the enrichment of the sequentially debulked samples on the MACSQuant Tyto Cell Sorter, expression of $CD4^+CD25^+CD127^{dim/-}$ cells were plotted and calculated. The sequential debulking step generated a total of 8.84×10^6 Tregs, demonstrated a sorting efficiency of 48% and an enrichment from 38% to 83% of target cells (data not shown). The subsequent purity sort on the MACSQuant Tyto Cell Sorter generated a total of 4.58×10^6 Tregs with a sorting efficiency of 61%. Final enrichment of $CD4^+CD25^+CD127^{dim/-}$ cells resulted in 98% (fig. 2). The total workflow on the MACSQuant Tyto Cell Sorter successfully enriched $CD4^+CD25^+CD127^{dim/-}$ Tregs from 38% to 98% purity in under 5 hours.

Results of the 14-day cell expansion were assessed by plotting CD25 vs FoxP3 expression from samples obtained before and after cell expansion. The cell expansion protocol successfully enriched $FoxP3^+$ cells from 92.7% in the pre-expansion sample to 98.3% in the post-expansion sample. The total number of $FoxP3^+$ cells increased from 4.5×10^6 cells to 41.4×10^7 cells post-expansion, representing a tenfold increase with 98.3% purity (fig. 3).

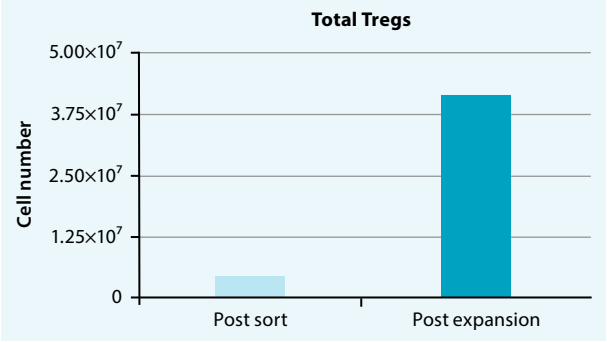


Figure 5: Expansion of Tregs. Sorted Tregs were expanded in culture for 14 days, stained with CD25-PE and FoxP3-FITC antibodies and analyzed with a MACSQuant Analyzer 10.

Conclusion

These data demonstrate successful isolation and enrichment of $CD4^+CD25^+CD127^{dim/-}$ Tregs in a closed system with the CliniMACS Prodigy and MACSQuant Tyto workflow. Our results demonstrated:

- Despite an extremely low percentage of target cells in the starting material, we achieved up to 98% purity.
- The CliniMACS Prodigy and MACSQuant Tyto processed approximately 2.5 billion cells and generated a total yield of 4.58×10^6 $CD4^+CD25^+CD127^{dim/-}$ Tregs.
- Following enrichment and sorting, $FoxP3^+$ cells were successfully expanded tenfold and maintained a high purity of over 98%.
- With single use, the MACSQuant Tyto Cartridge HS (High-Speed), Tregs were enriched from 38% to 98% in under 5 hours.

Beyond successful and rapid sorting of Tregs, the single-use, disposable, and high-speed cartridges offer safety and ease of use. The operator is not exposed to patient samples in the form of aerosols and cells can be transferred directly from the sterile cartridges into antibiotic-free culture and successfully expanded.

The MACSQuant Tyto also offers a comprehensive workflow for clinical isolation of Tregs including the MACS GMP Tyto Cartridge, MACS GMP Tyto Running Buffer, and MACS GMP Fluorescent Antibodies.

With the easy MACSQuant Tyto workflow and the rapid sorting of the MACSQuant Tyto Cartridge HS, the next breakthrough in clinical research of Tregs could be just around the corner.

References

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Products

Product	Order no.
CliniMACS Prodigy	200-075-301
CliniMACS Prodigy TS 510	170-076-629
CliniMACS Anti-Biotin Reagent	170-076-709
MACS GMP ExpAct Treg Kit	170-076-119
MACS GMP Recombinant Human IL-2, (500 µg)	170-076-147
Treg Expansion Kit, human	130-095-345
TexMACS GMP Medium, 2000 mL	170-076-306
MACS GMP CD4-VioBlue Fluorescent Antibodies	170-076-504
MACS GMP CD25-PE-Biotin Fluorescent Antibodies	170-076-505
MACS GMP CD127-APC Fluorescent Antibodies	170-076-501
MACSQuant Tyto Cell Sorter	130-103-931
MACSQuant Tyto Cartridge HS	130-121-549
MACSQuant Tyto Running Buffer	130-107-206
MACS GMP ExpAct™ Treg Kit	170-076-119
MACS GMP Rapamycin	170-076-308



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Miltenyi Biotec B.V. & Co. KG | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macsde@miltenyi.com | **www.miltenyibiotec.com**
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