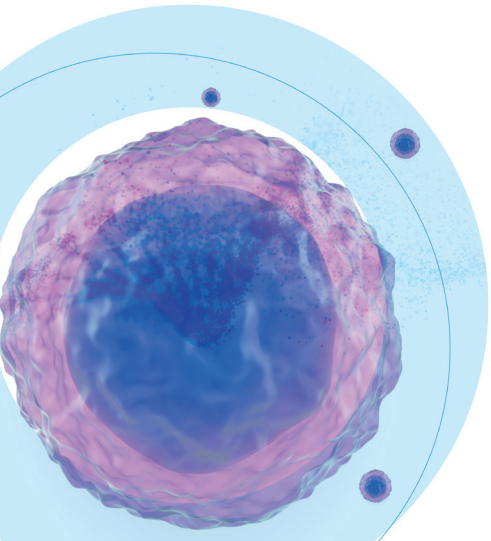
Isolation Of Highly Pure and Viable T and NK Cells Using the CGX10 Cell Isolation System

Strategies for harnessing the immune system to combat cancer and autoimmune diseases using immunotherapy represent a promising approach in personalized medicine. In this context, many efforts are emerging for developing and engineering T cell and NK cell–based immunotherapy. Preclinical studies demonstrate the success of using these immune cells in hematological and other malignancies.

The most employed cell separation platform for large-scale enrichment and isolation of T and NK cells for cell therapy is magnetic-assisted cell separation, which separates cells based on a single surface antigen. Enrichment based on multiple cell surface markers or different expression levels of these markers often requires the use of a traditional cell sorter. This application note describes the use of the CGX10 Cell Isolation System, a closed system suitable for GMP–compliant cell separation. The CGX10 Cell Isolation System utilizes a novel microfluidics technology to achieve high purity cell isolation within a closed and sterile microfluidics chip. The purity and viability of regulatory T cells, naïve T cells, and NK cells purified by the CGX10 Cell Isolation System were compared to those obtained with a traditional cuvette cell sorter.

Materials and Methods

Frozen human peripheral blood mononuclear cells (IQB-PBMC103) were thawed and stained with the antibodies shown in the following tables to identify regulatory T cells, naïve T cells, and NK cells. Propidium iodide (PI) was used to check for viability after cell isolation analysis.



Regulatory T–Cell Reagents

Antibody/Dye	Product name	Product number	Manufacturer
CD4/Pacific Blue™	Anti-human CD4, Human (Mouse) – Pacific Blue™	300521	BioLegend
CD45RA/FITC	Anti-human CD45RA, Human (Mouse) – FITC	304106	BioLegend
CD25/PE	Anti-human CD25, Human (Mouse) – PE	302606	BioLegend
CD127/APC	Anti-human CD127, Human (Mouse) – APC	351316	BioLegend

Naïve T–Cell Reagents

Antibody/Dye	Product name	Product number	Manufacturer
CD4/Pacific Blue™	Anti-human CD4, Human (Mouse) – Pacific Blue™	300521	BioLegend
CD45RA/FITC	Anti-human CD45RA, Human (Mouse) – FITC	304106	BioLegend
CD62L/PE	Anti-human CD62L, Human (Mouse) – PE	304806	BioLegend
CD8/APC	Anti-human CD8a, Human (Mouse) – APC	301014	BioLegend

NK–Cell Reagents

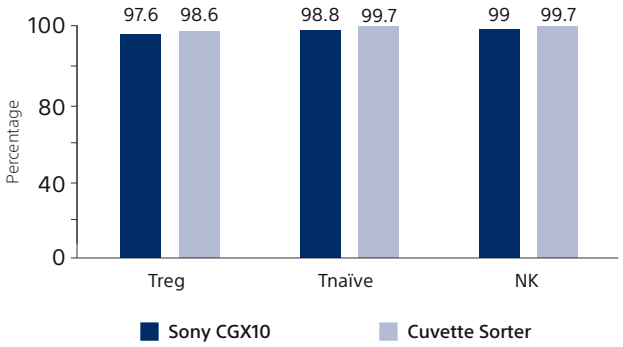
Antibody/Dye	Product name	Product number	Manufacturer
CD19/Pacific Blue™	Anti-human CD19	302232	BioLegend
CD16/PE	PE Mouse Anti-Human CD16 Clone 3G	555407	BD Biosciences
CD3/FITC	Anti-human CD3, Human (Mouse) – FITC	300406	BioLegend
CD56/APC	Anti-human CD56, Human (Mouse) - APC	362504	BioLegend

Samples were concentrated to $2x10^7$ cells/mL and resuspended in PBS (Mg+) containing Pluronic® F-68 (0.1%), HSA (0.5%), and DNase (150 U/mL), aliquoted into two tubes, and run on the CGX10 and a traditional cuvette cell sorter with concentration adjustment only for the cuvette cell sorter. Doublets were gated using scatter signals (FSC, SSC/BSC), and CD4+CD45RA+CD25+CD127- (regulatory T cells), CD4+CD8-CD62L+CD45RA+ (naïve T cells), and CD3-CD56+CD16+CD19- (NK cells) were gated for sorting. Isolation performance from the CGX10 Cell Isolation System was compared to sorting results of the same samples run on a traditional cuvette cell sorter.

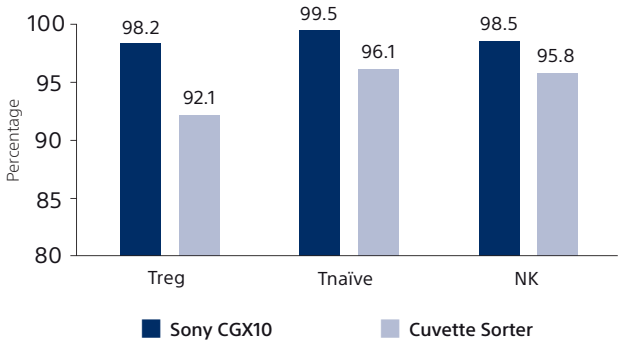
Results

Post-isolation purity of cells run on both the CGX10 and cuvette sorter showed high purity, over 97% (A). Purity was calculated after doublet gating, not shown in figures. Post-isolation cell viability results from the CGX10 showed higher viability than those from the cuvette sorter in all three cell types, regulatory T cells, naïve T cells, and NK cells (B). Regulatory T cells were gated with CD4+CD45RA+CD25+CD127- (C). The CGX10 Cell Isolation System post-isolation result (D) showed equivalent high purity compared to the cuvette sorter post-cell isolation result (E). Naïve T cell and NK cell plots are not shown. All data were measured by a commercial cell analyzer and the FCS files were analyzed using a commercially available data analysis software.

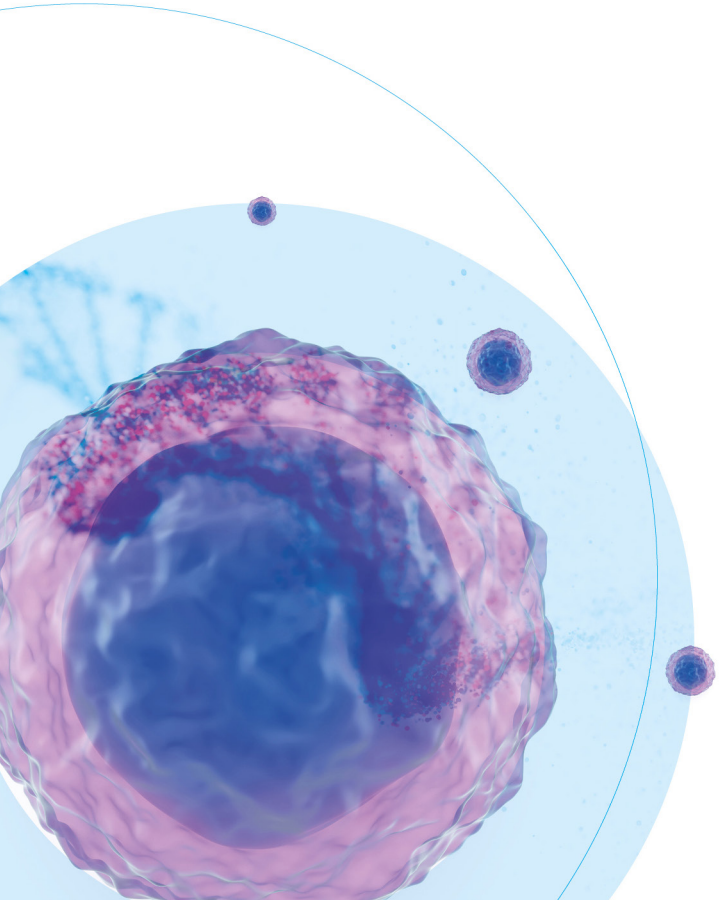
A) Purity



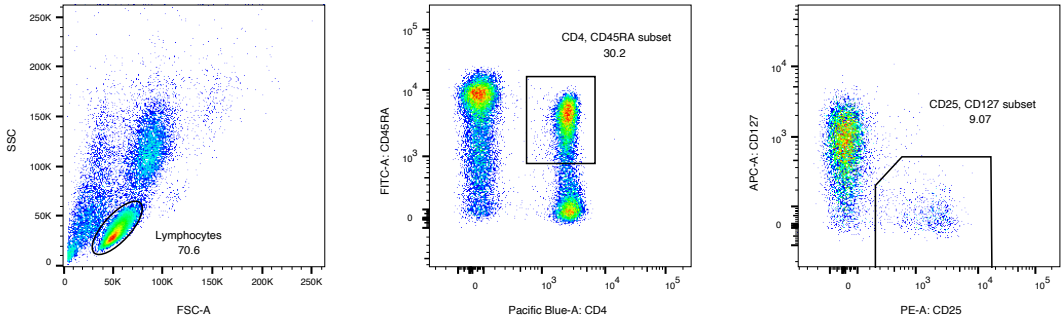
B) Viability



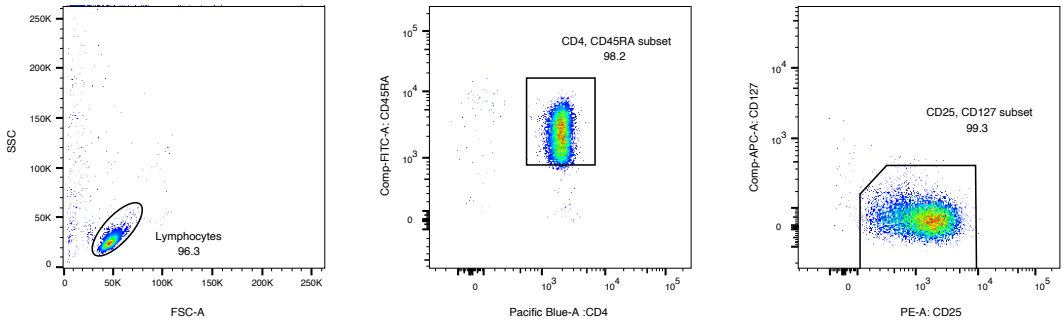
(A) Post-isolation purity of cells run on both the CGX10 and cuvette sorter.
(B) Post-isolation cell viability comparison between the CGX10 and cuvette sorter in three cell types, regulatory T cells, naïve T cells, and NK cells.
(C) Regulatory T cells sorting gated with CD4+CD45RA+CD25+CD127-
(D) Regulatory T cells post-isolation result with CGX10 Cell Isolation System.
(E) Regulatory T cells post-isolation result with cuvette sorter.



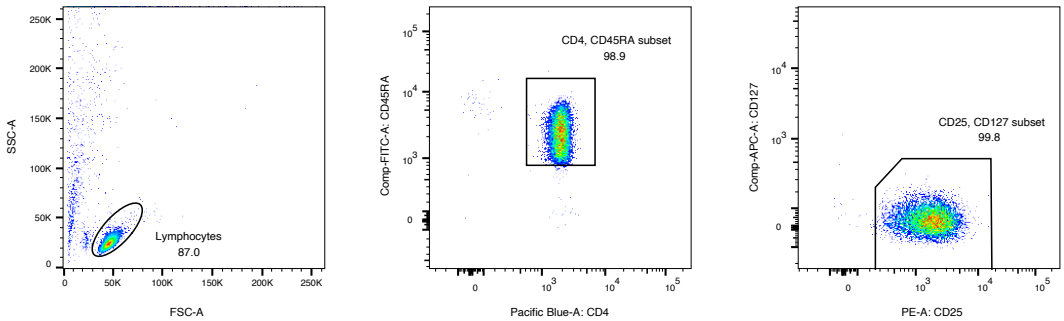
C) Pre-sorting (regulatory T cells)



D) CGX10 post-sorting (regulatory T cells)



E) Cuvette sorter post-sorting (regulatory T cells)



Event Rate (Eps)

	Regulatory T cells	Naïve T cells	NK cells
CGX10	14,000	16,500	10,000
Cuvette Sorter	7,800	6,500	9,300

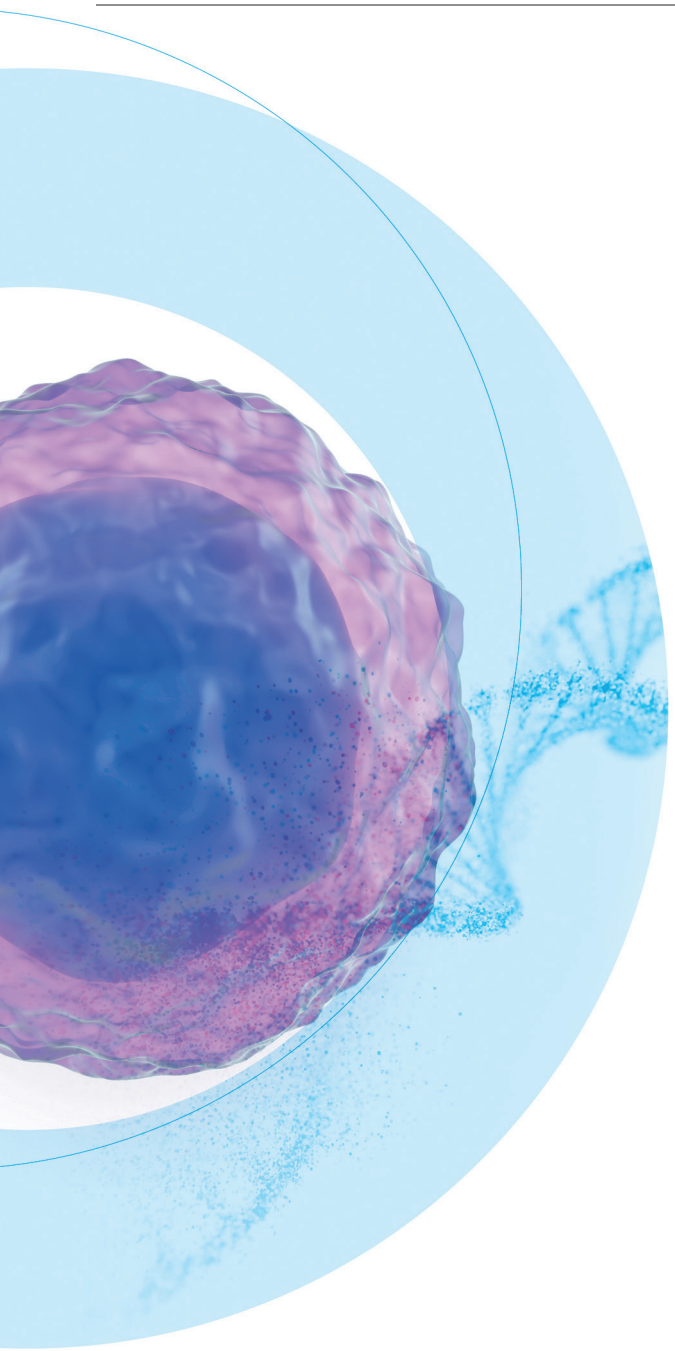
Event Rate (Eps) comparison between from the CGX10 Cell isolation system and cuvette sorter in three cell types, regulatory T cells, naïve T cells, and NK cells.

Summary

In this application note we highlight the capability of Sony's CGX10 cell isolation system using microfluidics based closed cell sorting technology to isolate target cell populations used in cell therapy protocols. The results demonstrate that the CGX10 can isolate cell fractions present in low and abundant levels at high throughput. The results also indicate that the CGX10 Cell Isolation System is able to deliver highly pure and viable cell subsets and outperforms a traditional cuvette sorter.

Acknowledgment

Sony Corporation is grateful to Dr. Hiroshi Shiku and Mie University for their collaboration in generating this data.



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The CGX10 Cell Isolation System and related products are intended for use by trained laboratory technicians in research, process development or manufacturing environments all related to ATMP/regenerative medicine, including cell and gene therapy. The CGX10 instrument and related products are for *ex vivo* cell separation processing only, and are not intended for therapeutic, diagnostic, or human *in vivo* applications. Any clinical application of the cells is exclusively within the responsibility of the user of the CGX10 instrument and related products. For the manufacturing and use of cells in humans, regulations must be followed. The CGX10 Cell Isolation System and related products are not sold as medical devices.

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