ISCT NA 2023

Development of a xeno-free electroporation buffer for large-scale electroporation

Akshaya Chandrasekaran[&], Khushboo Pathak[&], Monique LaCourse[&], Gregory Bonello[&], Namritha Ravinder[&]
[&]Thermo Fisher Scientific, 5781 Van Allen Way, Carlsbad, CA 92008.

Abstract

Electroporation (EP) is emerging in the cell and gene therapy space as a safe, effective, and promising non-viral strategy to engineer chimeric antigen receptor (CAR)-T cell. T cell exhaustion has been recognized to play an immunosuppressive role in cancer and is a primary cause of resistance to CAR-T cell therapies. Accumulating evidence in mice and humans suggests that T cell differentiation negatively correlates with long-term anti-tumor activity, with early memory T cells allowing for greater and persistent anti-tumor effects. A review of early CAR-T trials of patients with less remission showed they had higher amounts of naïve and stem memory T cells in starting material. These naïve resting T cells are non-dividing cells which renders them difficult to transfect by EP. We developed a xeno-free low conductivity buffer formulation that will enable higher energy settings on the CTS™ Xenon™ Electroporation System to expand Xenon optimization capabilities for difficult cell types such as resting T cells.

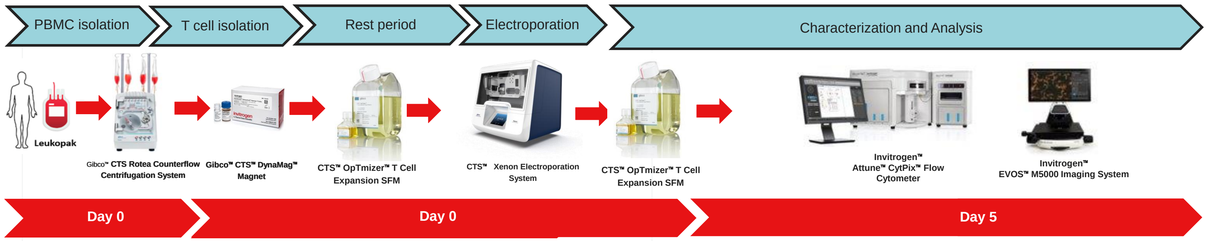
We use an automated counterflow centrifugation system to isolate peripheral blood mononuclear cells from fresh leukopaks and perform downstream T cell isolation. Electroporation reactions are set up and optimized on the Neon, Neon™ NxT, and Xenon Electroporation Systems and phenotyping is performed pre- and post-EP to monitor the stemness of the resting T cell population using flow cytometry. Transfection efficiency and cell viability are measured by flow cytometry and resting T cell phenotype is monitored for maintenance of stemness. Preliminary results show that the buffer successfully delivers mRNA and plasmid payloads into resting T cells using higher voltage settings on at efficiencies consistently higher than those observed from using the Neon Resuspension Buffer R. This is also accompanied by the maintenance of resting T cell phenotype as measured by the expression of stemness and activation markers. Ongoing work includes optimization and functional testing of the xeno-free buffer for gene editing applications including KO/KI. Thus, the xeno-free buffer will provide a wider design space and the opportunity to further optimize EP reactions for process development and commercial manufacturing.

Introduction

Thermo Fisher Scientific has developed a lower conductivity buffer formulation for the CTS™ Xenon™ Electroporation System that can enable higher energy settings to be applied to cells without adversely affecting viability. This solution aligns Xenon™ buffer offerings to Neon™ NxT Electroporation System and expands Xenon™ utility to other cell types and applications. This product will provide manufacturers a cell therapy-ready formulation in formats that allow for scalability and closed modular processing.

Materials and Methods

Human PBMCs were isolated from a fresh Leukopak using the CTS Rotea™ System. T cells were isolated using Dynabeads™ Untouched™ Human T Cells Kit and CTS™ DynaMag™ magnet. Following isolations, cells are allowed to rest in Gibco™ CTS™ OpTmizer™ T Cell Expansion SFM, supplemented with Gibco™ CTS™ Immune Cell SR, CTS™ GlutaMAX™, IL-7, and IL-15 for 3 hours. After the rest period, cells were washed using DPBS (without Ca^{2+} and Mg^{2+}), supplemented with 1% HSA Human Serum Albumin and 1mM EDTA. Post-wash, cells were resuspended in CTS™ Xenon™ Lower Conductivity Electroporation Buffer. For mRNA and plasmid transfections, EGFP mRNA and emGFP plasmid (Aldevron) were used. For TCR knock-out (KO) using the CTS™ Xenon™ system, CTS™ TrueCut™ Cas9 Protein was used in conjunction with TrueGuide™ Synthetic gRNA. Following electroporation, T cells were transferred to Gibco™ CTS™ OpTmizer™ T Cell Expansion SFM, supplemented with Gibco™ CTS™ Immune Cell SR, CTS™ GlutaMAX™, IL-7, and IL-15 and cultured for up to 10 days without CD3/CD28 activation. The cells were supplemented with fresh media every 3 days. Post-electroporation, cells were activated using CTS™ CD3/CD28 Dynabeads™ for 48 hours and de-beaded prior to flow cytometry analysis. The cells were evaluated for viability, transgene expression, and maintenance of stemness markers. Prior to large-scale, small-scale electroporation optimization was carried out using Invitrogen™ Neon™ NxT Electroporation System.



Results

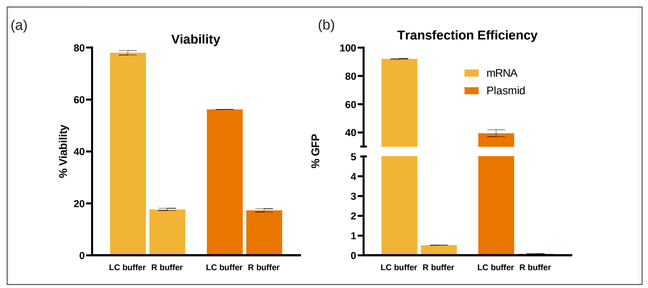


Figure 1. LC buffer outperforms R buffer in Resting T Cells. CTS Lower Conductivity Electroporation Buffer outperforms CTS Electroporation Buffer for both mRNA and DNA based payloads in resting T cells as observed from a) cell viabilities post-electroporation, and b) % GFP mRNA or plasmid transfection efficiency using Neon Resuspension Buffer R or CTS Lower Conductivity Electroporation Buffer on Xenon electroporation system.

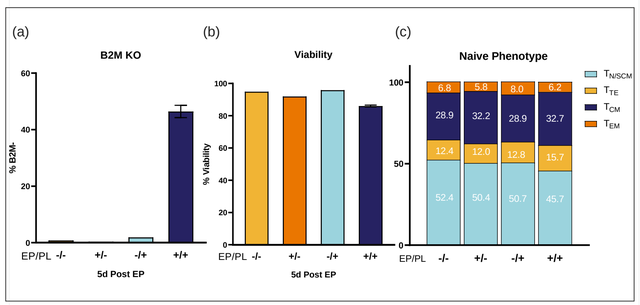


Figure 2. LC buffer demonstrates successful Cas9 RNP delivery in resting T cells. a) The CTS Xenon Lower Conductivity Electroporation Buffer demonstrates successful delivery of CTS™ TrueCut™ Cas9 Protein and TrueGuide B2M Synthetic gRNA using higher energy settings on the Xenon electroporation system as indicated by b) maintenance of cell viability 5 days post-electroporation compared to controls, and c) maintenance of % stemness marker expression after electroporations using CTS Lower Conductivity Electroporation Buffer.

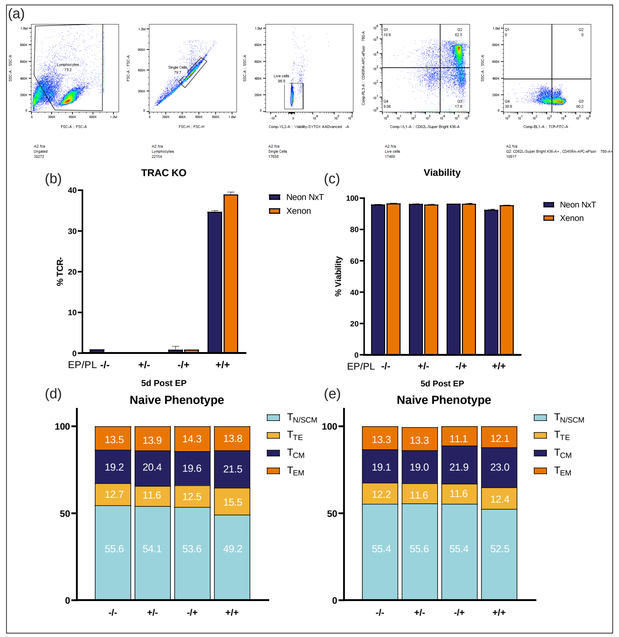


Figure 3. LC buffer demonstrates scalable Cas9 RNP delivery in resting T cells Neon NxT to Xenon electroporation system. a) Resting T cells were stained to verify CD45RA and CD62L surface expression markers and analyzed for TCR expression 5 days post-electroporation using flow cytometry. b) The CTS Xenon Lower Conductivity Electroporation Buffer demonstrates successful and scalable delivery of CTS™ TrueCut™ Cas9 Protein and TrueGuide TRAC Synthetic gRNA using higher energy settings on the Xenon electroporation system compared to RNP delivery using Neon NxT Resuspension Buffer T on the Neon NxT system as indicated by c) maintenance of cell viability 5 days post-electroporation compared to controls, and maintenance of % stemness marker expression after electroporations using NxT Resuspension Buffer T (d) and CTS Lower Conductivity Electroporation Buffer (e).

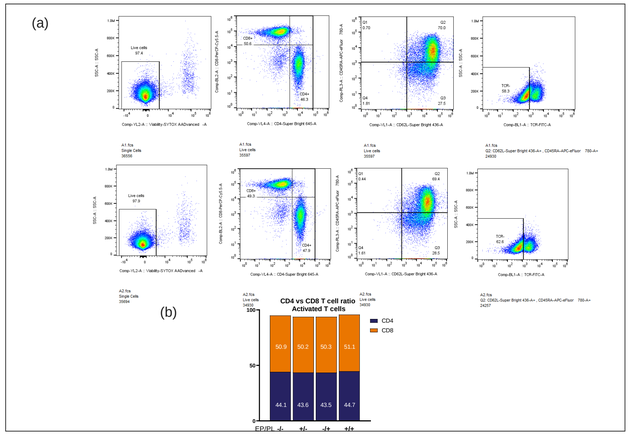


Figure 4. Activation post-electroporation for downstream analysis. a) Resting T cells can be successfully activated using CTS CD3/CD28 Dynabeads for 48 hours and de-beaded prior to flow cytometry analysis for stemness markers and analysis of CD4 and CD8 T cell ratios. b) Proportions of CD4 and CD8 T cells remained largely consistent from non-electroporated cells to cells electroporated with CTS™ TrueCut™ Cas9 Protein and TrueGuide TRAC Synthetic gRNA using the CTS Xenon Lower Conductivity Electroporation Buffer.

Conclusions

CTS Lower Conductivity Electroporation Buffer allows application of higher energy settings on the Xenon electroporation system without the marked increase in sample temperature seen with other buffer formulations. This enables an expanded range of combinations of voltage, pulse width, and pulse number for customers and allows cell therapy developers to optimize electroporation processes with a greater chance of improved viability, transfection efficiency, and total number of modified cells at the end of their manufacturing processes. More specifically, this buffer can benefit difficult to transfect cell types and applications such as heat-sensitive cells or transfections at lower cell densities.

Acknowledgements

We would like to thank everyone involved in this project including Don Paul Kovarcik, Nektaria Andronikou, Pushpalatha Chaluvappa, Sarah Coleman, Shahan Molla, and Arseny Smirnov, for their help and support.

Science at a scan
Scan the QR code on the right with your mobile device to download this and many more scientific posters.

Learn more at https://www.thermofisher.com/celltherapy