CAR-T cell therapy manufacturing with the ekko™ System: DMSO removal for thawed apheresis material

Application Note

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Key Words: T cells, ekko™, acoustic, CAR-T, apheresis, leukopak, cell therapy

Apheresis material DMSO removal

Background

Chimeric Antigen Receptor T cell (CAR-T) therapies have spearheaded a renaissance in immunology and regenerative medicine. As these therapies gain approval, bottlenecks are moving from the clinic to the manufacturing site. Here, manufacturing technologies that provide consistent, closed, and automated solutions to unit operations are required to enable robust and affordable therapeutic production.

Processing apheresis material using ekko™ System

For CAR-T processes that require a freezing step between apheresis collection and manufacturing, it is critical upon thaw to remove DMSO, a standard cryoprotectant, from the product to ensure the utmost cellular health of the apheresis material. The ekko™ system enables user-friendly automation of wash and concentration steps. The ekko™ system is a functionally closed, integrated manufacturing platform which gently processes cells supporting removal of DMSO and cell debris.

ekko™ System Overview

The ekko™ system is a GMP capable platform technology for cell and gene therapy manufacturing. The system uses acoustophoresis to hold cells at low energy locations in a three-dimensional standing wave based on their size, density and compressibility. As acoustic technology provides a wide operating window, the ekko™ can be used and optimized for a variety of unit operations throughout the production workflow, including but not limited to concentration and/or wash. Intuitive controls and a purpose-built single-use cartridge make the ekko™ system a flexible and scalable tool for early stage research through to GMP production.

Experimental Setup

The ekko™ system is sterile-welded to the cell source via industry standard PVC and C-Flex tails to enable connection to a variety transfer bags.

Apheresis washing processes require two (2) connections between the transfer bag containing the input material and ekko™ system. Due to the deleterious effects of DMSO on cells, it is recommended that apheresis material be processed as soon as possible once thawed. Initial dilution of the apheresis material with wash buffer is performed to further minimize DMSO exposure during processing.

Figure 1. ekko™ Acoustic Cell Processing System.

Media Exchange Process Steps

1. Prime ekko™ chamber & dilute feed material with fresh media
2. Recirculate input material through ekko™ chamber with acoustics ON, seeding acoustic wave with cells
3. Concentrate cells in ekko™ chamber, flowing supernatant to waste
4. Wash cells in ekko™ chamber with fresh media
5. Collect washed cells in product bag at specified volume

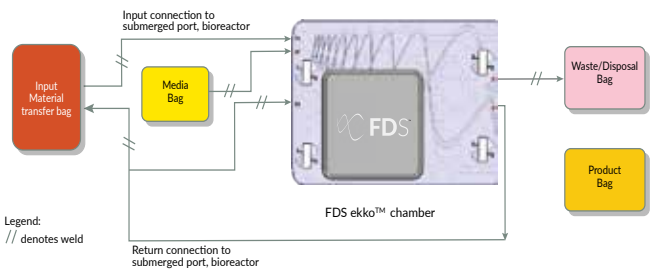

Figure 2. Process Flow Diagram for thawed apheresis DMSO washout procedure using ekko™ system.

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Process Development Method

Apheresis material varies greatly from patient-to-patient, the ekko™ system provides a flexible platform that can be optimized to give consistent performance on every patient. Performance is based on the length of the recirculation step, and ratio of acoustic power and flow rate. Process values can be determined empirically by performing power and recirculation time titration and measuring the waste stream. It is recommended that these experiments be performed at the lower limit of expected patient cell number to ensure that robust protocols are developed.

Materials & Methods

Frozen apheresis products were thawed and diluted to 500ml (nominal) with TexMACS (Miltenyi) media. 10ml manual centrifugation controls were run in parallel to ekko™ processing, comparing product recovery and viability. To model cell performance in downstream-processing, products from ekko™ and centrifuge were activated with Dynabeads® (3:1 bead to cell ratio) and cultured for seven (7) days in a twenty-four (24) well plate system (triplicate 1 million mononuclear cells per sample). Cell counts and viability determined using Nucleocounter NC-200. Cell phenotype determined using flow cytometry.

Results

Washes of thawed apheresis product showed consistent recovery between multiple samples and donors. No significant changes in phenotype and expansion potential were seen between ekko™ processing and manual centrifugation.

Conclusion

The ekko™ Acoustic Cell Processing System is a closed, automated, and consistent platform for processing apheresis products and allows for:

- Repeatable process outputs across multiple donors and samples
- Tunable protocols enable large operating with regard to input cells
- High recovery of mononuclear cells, with no impact to phenotype or expansion potential

Table 1. Result of n=4 (donor n=2) apheresis washes. NM= Not Measured.

Process Inputs	ekko™ processing	Manual Centrifugation
Volume (mL)	475±3	9.97±0.04
Total Mononuclear Cells (e9 cells)	1.8±0.5	0.042±0.011
Cell Viability (%)	93±1%	92±1%
Process Outputs		
Volume (mL)	85±2	2.13±0.02
Total Mononuclear Cells (e9 cells)	1.6±0.4	0.037±0.010
Cell Viability (%)	91±2%	94±1%
Process Performance		
Viable Cell Recovery (%)	89±3%	87±4%
Process Time (minutes)	41.9±0.1	NM
Viability Change (%)	-1±1%	1±2%

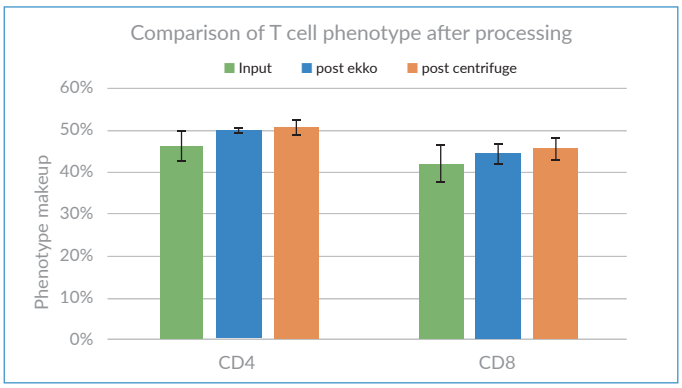

Figure 3. Comparison of T cell helper (CD4) and cytotoxic (CD8) populations post ekko™ and manual centrifugation processing. Similar phenotypic breakdown seen in all samples.

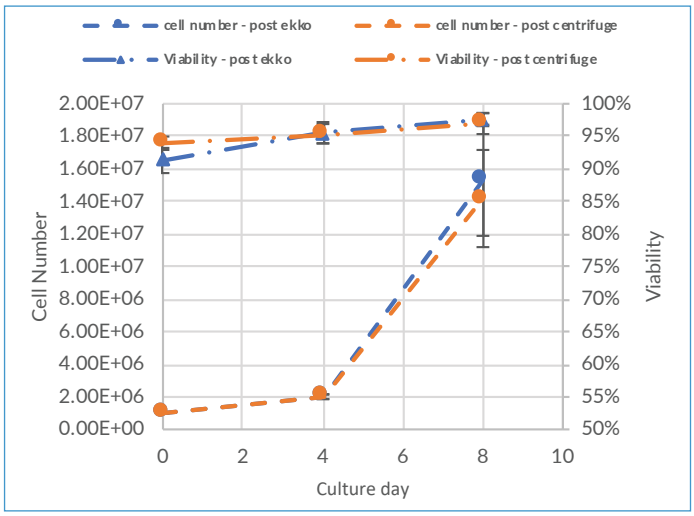

Figure 4. Comparison of T cell expansion post ekko™ and manual centrifugation processing. Similar expansion potential seen after both processes.

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The ekko™ acoustic cell processing system is GMP capable in its design. It has not been validated for any particular process. For applications requiring regulatory submissions, users may request supporting documentation from FloDesign Sonics to support their filing.

See operator's manual for additional information.

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