Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

Acoustic Cell Processing with ekko™ System

How it Works

Introduction

The ekko™ Acoustic Cell Processing System is an automated and closed platform designed to support unmatched flexibility and ease of use for wash, concentration, and media exchange applications. The system is designed for cell therapy organizations focused on research programs through manufacturing, along with those in need of an industrialized, adaptable cell production system uniquely capable of the gentle, scalable, and reproducible processing required to achieve the promise of advanced therapies.

The ekko™ Acoustic Cell Processing System frees users of traditional mechanical technologies, increasing cell quality. With ekko™ Architect, our protocol development tool, total control is at your fingertips to help support your program goals.

Fit-for-Purpose Cell Therapy Processing Platform

Concentrate and wash steps throughout the cell therapy process can be particularly challenging for various reasons – low cell recovery and viability, open-processing steps, and operator error can plague this unit operation. The ekko™ System overcomes the challenges observed with alternative options like mechanical and filtration-based methods by employing acoustic technology to gently process cells. This method results in safe and efficient manufacturing with improved economics and process freedom while delivering reproducible results.

The ekko™ Acoustic Cell Processing System is flexible for a range of cell types and variations including without limitation to:

Process Description	Use Case
Wash and media exchange	Previously cryo-preserved apheresis material DMSO wash-out
	iPSC cell culture media exchange
	Formulation
Concentration	T cell harvest from small volume expansion vessel
	MSC harvest from mid-scale expansion vessel
	HSC harvest
Low Volume Processing	T cell genetic modification preparation
	Sample preparation for analysis
Separation	iPSC aggregate processing and single cell removal

MilliporeSigma

What is acoustophoresis?

Acoustophoresis means migration (-phoresis) with sound (acousto-). Acoustophoresis is a noncontact, or touchless, methodology for manipulating cells and particles, which allows for various uses. The scattering of the sound wave off a suspended particles creates a steady acoustic radiation force on the particle. The force scales with particle and fluid density and compressibility. When the acoustic radiation force exceeds the combined effect of fluid drag force and gravity/buoyancy, the particle moves to its stable location, where it is trapped in the acoustic field. The acoustic field structure and secondary interparticle forces result in the formation of particle or cell clusters. Enhanced gravitational settling takes place when the clusters reach a critical size or when the acoustic field and flow is turned off.

With acoustophoresis, the predominant force acting on cells in the acoustic wave is the primary axial acoustic radiation force **(Figure 1)**. Cells are suspended in medium by using acoustic forces generated by sound waves through the medium. When exposed to an acoustic force, particles in suspension will move in the sound field if the acoustic properties of the particle differ from the surrounding medium. The magnitude and direction of response depend on factors like:

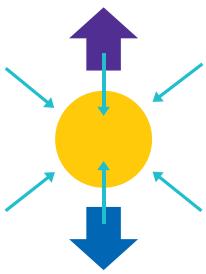
- **Particle size, density, and compressibility**
- **Acoustic power and frequency**
- **Liquid medium density**

Besides the primary axial radiation force, secondary forces act on cells in a wave **(Figure 1)**. These relate to the inter-cell forces generated by the proximity of cells to one another and assist in the formation of loose clusters. These clusters consist of disparate cells and separate once the acoustic force is turned off or the cluster settles through gravity and separates.

Figure 1: Together, these forces influence the ability to capture, settle, or otherwise manipulate cells using acoustophoresis

Fluid Drag Force

- Fluid and cell velocity
- Dynamic viscosity of fluid



Acoustic Force

- Cell and fluid density and compressibility
- Cell volume
- Acoustic field gradients

Gravitational Force

- Cell or cluster size
- Cell and fluid density
- Dynamic viscosity of fluid

How is Acoustophoresis used in the ekko™ System for Cell Therapy Manufacturing?

Our technology employs a complex standing wave field generated in the ekko™ Single Use Assembly's Acoustic Chamber to manipulate cells. A piezoelectric transducer, which converts electrical charges into mechanical energy, is located on the ekko™ System's Fluid Handling Unit. The transducer launches forward propagating waves which are reflected and diffracted by the faceted reflector opposite to the transducer and located in the ekko™ Single Use Assembly. Our faceted reflector increases the acoustic field gradients and enhances particle clustering. This innovation increases acoustic field stability and enables high cell retention and concentration. As cells flow into the acoustic chamber, the acoustic radiation force captures the cells, which migrate towards areas of low acoustic pressure amplitude called the nodes. As additional cells enter the chamber, they form loose clusters **(Figure 2)**. Buffer is passed through the acoustic chamber to wash the captured cells. These cell clusters, through gravity, gently settle out of the acoustic channel into the collection reservoir. Alternatively, the acoustics can be turned off and the cells collected upon settling **(Figure 3)**.

With acoustic technology, processes can be easily optimized to achieve required performance and control. Furthermore, adjustments to parameters like acoustic power and flow rate, factors that influence the acoustic radiation force and drag force, allow for simple yet effective procedural enhancements which makes the ekko™ Acoustic Cell Processing System a robust manufacturing tool.

Figure 2: Acoustic wave with node clustering

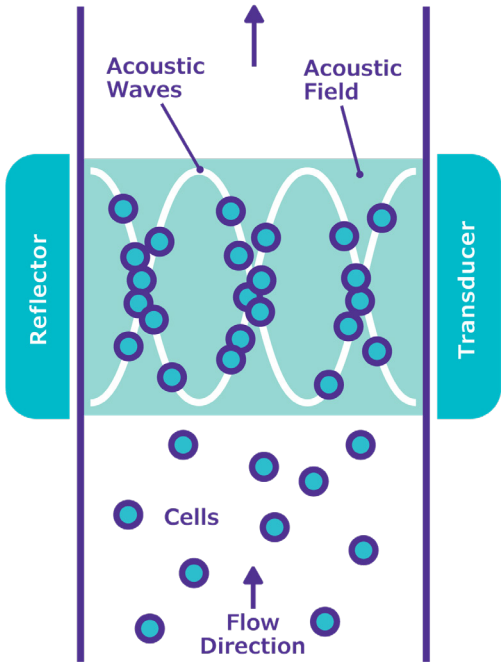
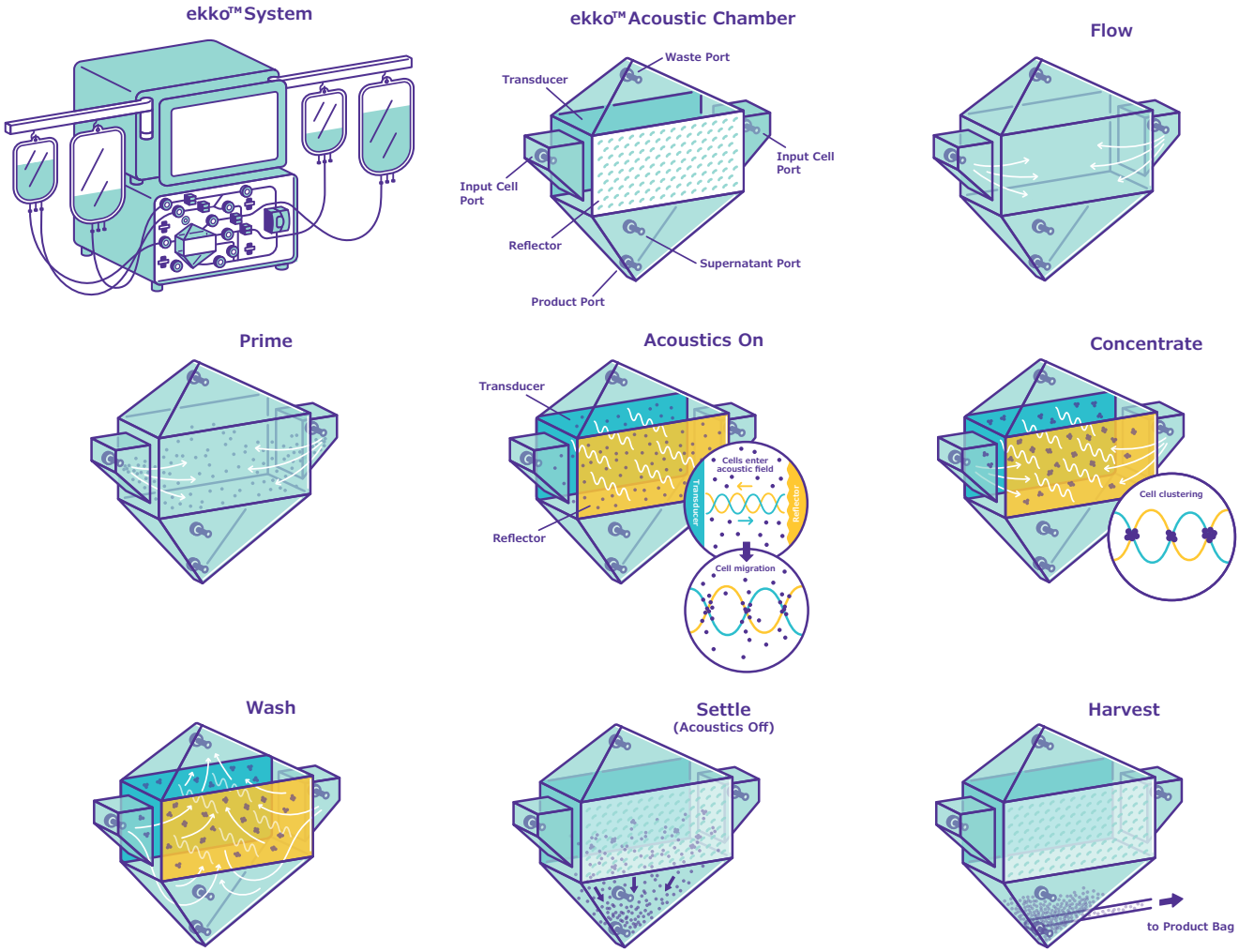


Figure 3: Acoustic process steps and flow path inside the acoustic chamber



ekko™ System Delivers Gentle Cell Processing

The ekko™ System employs acoustic technology to provide gentle processing of valuable cells used for manufacturing. The ekko™ System has been extensively analyzed to evaluate shear stress exposure to cells processed with the platform. All sources of potential shear exposure have been interrogated, including:

- **Shear generated by flow,**
- **Shear generated by the acoustic field on a single cell migrating into the acoustic chamber,**
- **Shear generated on a single cell captured by the acoustic chamber, and**
- **Shear generated on a cell located in a cluster of cells in the acoustic chamber**

Using Computational Fluid Dynamics (CFD) simulations in COMSOL, with fluid modeled as water at a viscosity of 0.001 Pa. s at 25°C (as cell culture fluids are nearly identical), studies show the effect on cells is benign. The primary source of shear is generated by the flow through the barbs of the inlet and outlets ports of the Acoustic Chamber, which reach maximum values of 10 dynes/cm^2 at flow rates of 45 mL/min, which is commonly used on the ekko™ System. This shear is lower than what is typically experienced in the human body's vascular system and much less than the critical shear stress values reported in literature. Furthermore, as acoustic waves are a pressure-based phenomenon, acoustic processing is essentially shear-free.

Empirical testing of T cells pre- and post- acoustic processing against centrifuge controls also revealed no impact on cell viability, expansion potential and phenotype after exposure.

The quality of acoustically or manually washed apheresis material and expanded T cells was assessed by monitoring cell growth in culture for 7 days post processing. Apheresis material from two donors underwent automated wash on the ekko™ System, or manual processing via centrifugation followed by PBMC isolation using Ficoll-Paque (Cytiva®). Growth and viability was found to be equivalent for T cells isolated from acoustically and manually processed apheresis material **(Figure 4)**.

Expanded T cells were harvested and underwent further processing with the ekko™ System or centrifuge controls, and then cryopreserved prior to evaluation. Cryopreserved harvest material obtained from ekko™ System exhibited similar post thaw recovery, identity and phenotype **(Figure 5)** as the centrifuge control.

Figure 4 Expansion potential following ekko™ System processing shows comparable growth in T cells isolated from the ekko™ (System automated) and centrifuge (manual) methods. Data shown represents cell yield and viability across 7 days of culture with two distinct donors.

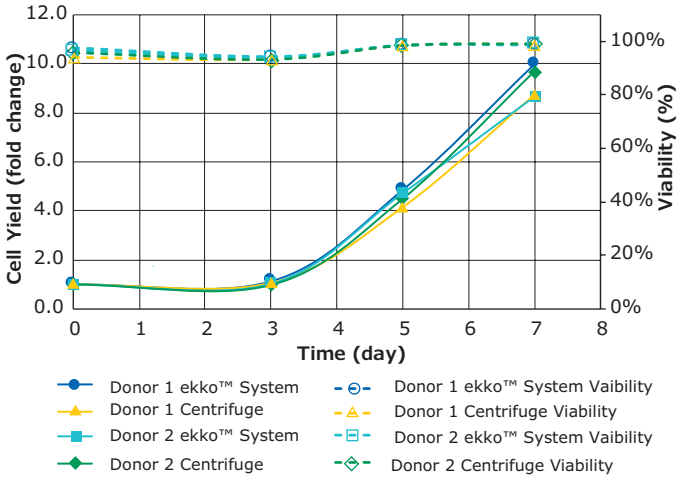
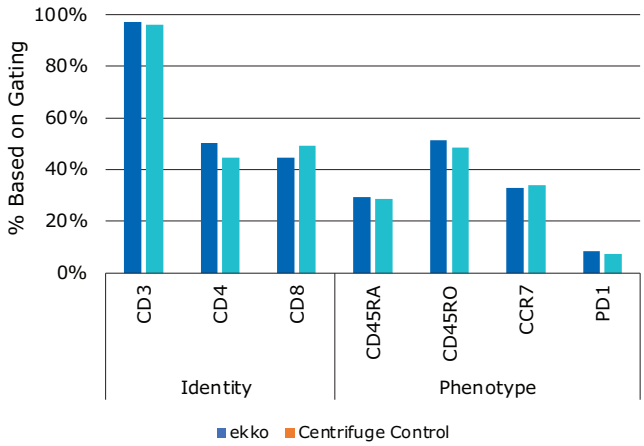


Figure 5: Expanded T cell (A) identity and (B) phenotype markers measured using flow cytometry.



Conclusion

Concentrate and wash steps throughout the cell therapy process can be particularly challenging for various reasons. The ekko™ System overcomes challenges observed with alternative options like mechanical- and filtration-based methods by employing acoustic technology to gently process cells. The ekko™ System results in safe and efficient manufacturing with improved economics and process freedom while delivering reproducible results.

Related Resources

- The ekko™ Acoustic Cell Processing System Specification Sheet, SS7577EN00
- Cell Therapy Manufacturing with the ekko™ Acoustic Cell Processing System: DMSO removal for Thawed Apheresis Material, AN5503EN00
- CAR-T Therapy Manufacturing with the ekko™ Acoustic Cell Processing System, AN6414EN00
- Integrated Pluripotent Aggregate Processing with the ekko™ Acoustic Cell Processing System, AN5434EN00.

Ordering Information

Ordering Information	
System	
ekko™ Instrument	**ACWHW001FXX***
Single Use Assembly	
ekko™ Single Use Assembly	**ACWSUF2001R**
ekko™ Single Use Maintenance Assembly	**ACWSUF2001RM**
Services	
Installation and Commissioning	**SSVQUAW001**
Preventative Maintenance	**SSVPRMW001**
Warranty	**SSVWTYW001**
Training	**SSVTRNW001**

*XX defined by region

References

1. M. Delahaye, K. Lawrence, S. J. Ward, M. Hoare, An Ultra Scale-Down Analysis of the Recovery by Dead-End Centrifugation of Human Cells for Therapy, Biotechnology and Bioengineering, Vol. 112, No. 5, May, 2015
2. J.P. Acosta-Martinez, I. Papantoniou, K. Lawrence, S. Ward, M. Hoare Biotechnology, Ultra Scale-Down Stress Analysis of the Bioprocessing of Whole Human Cells as a Basis for Cancer Vaccines, Biotechnology and Bioengineering, Vol. 107, No. 6, 2010
3. T. Papaioannou, C. Stefanadis, Vascular Wall Shear Stress: Basic Principles and Methods, Hellenic J Cardiol 46: 9-15, 2005

For more information on the ekko™ Cell Processing System, including additional documentation, videos, and animations please visit:

SigmaAldrich.com/ekkosystem

MilliporeSigma
400 Summit Drive
Burlington, MA 01803

To place an order or receive technical assistance

In the U.S. and Canada, call toll-free 1-800-645-5476
For other countries across Europe and the world, please visit: **EMDMillipore.com/offices**
For Technical Service, please visit: **EMDMillipore.com/techservice**

SigmaAldrich.com

MS_AN8435EN Ver. 1.0
37906
01/2022