

# VOLUME REDUCTION, CELL WASHING AND AFFINITY CELL SELECTION USING MULTI-DIMENSIONAL ACOUSTIC STANDING WAVE TECHNOLOGY

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Acoustic Cell Processing is a unique acousto-fluidics platform technology for shear-free manipulation of cells using ultrasonic standing waves. The platform has broad applications in the field of cell and gene therapy, e.g., cell concentration and washing, cell culturing, microcarrier/cell separation, acoustic affinity cell selection and label-free cell selection. The acoustic radiation force exerted by the ultrasonic standing wave on the suspended cells in combination with fluid drag forces and gravitational forces is used to manipulate the cells and achieve a certain cell processing unit operation, e.g., separate, concentrate, or wash. The technology is single-use, continuous, and can be scaled up, down or out. It therefore allows for a flexible and modular approach that can be customized to process a desired cell count, cell culture volume or cell concentration within a given required process time. Utilizing its proprietary multi-dimensional standing wave platform, FloDesign Sonics (FD Sonics) has been developing two applications for cell and gene therapy manufacturing, an Acoustic Concentrate-Wash (ACW) and Acoustic Affinity Cell Selection (AACS) system for closed and shear free Cell and Gene Therapy manufacturing, namely CAR-T immunocellular therapies. The ACW technology has been applied to Jurkat T-cells and primary cultures of T-cells of 1-2 Liters (L) with cell concentrations ranging from 1 million cells per milliliter (ml) to 40 million cells per ml. The process flow rate varies from 2-3 L/hour with average cell recoveries of more than 80% in 60 to 90 minutes. The efficiency of the cell washing process ranges from 95-99% depletion of a model protein (BSA), depending on the wash methodology. The AACS technology is a scalable acoustic affinity cell selection method using acoustic (non-paramagnetic) affinity beads for positive or negative cell selection. A multi-dimensional acoustic standing wave is then used to separate the affinity bead-cell complexes from the unbound cells, thereby completing the process of a negative or positive cell selection. A population of 1 billion CAR-T cells containing 30% T-Cell Receptor positive (TCR+) and 70% T-cell Receptor Negative (TCR-) cells has been depleted of 99% of its TCR+ population. The TCR- cell recovery for this process was above 70% and the full process took less than 2 hours. When used for positive selection of CD3+ cells, AACS allowed for an enrichment of 2.5-fold in CD3+ population. ACW and AACS are powerful acoustic-based cell processing technologies that lower cost and risk while enabling a modular, automation-friendly manufacturing process for cell and gene therapy manufacturing.

Robust viable cell recovery (VCR) from 1-2L and 1-40E6/mL

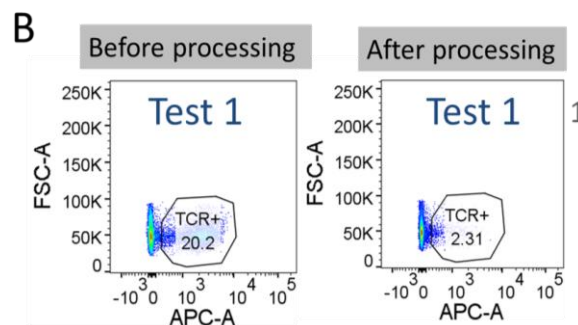
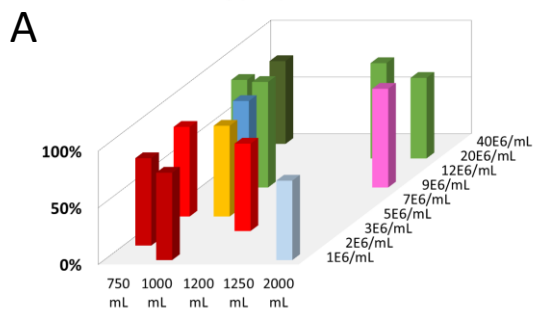


Figure 1 – Acoustic Concentrate Wash (ACW, A) and Acoustic Affinity Cell Selection performance (AACS, B). A depicts the cell recoveries obtained using ACW in the input space from 1-2L and 1-40 million cells per mL., using Jurkat T-cells; these results were confirmed with primary human T-cells. B shows a typical AACS TCR+ cell depletion result using one of FD Sonics proprietary acoustic beads, where 100 million primary human T-cells with 20% TCR+ were 10-fold depleted.