RESEARCH SNAPSHOT

Single Cell Enrichment Reveals Unaltered and Unbiased Results

Overview

Is there a way to feel confident that the data obtained is a true reflection of what's being studied? Conventional single-cell preparation methods tend to damage cells and reduce cell viability. This reduces the number of cells available for downstream experiments, but more importantly, the stress of multiple manipulations can change cellular phenotypes, transcriptional signatures of cells, or the makeup of the population being studied.

KEY HIGHLIGHTS

- Increase in cell viability from ~20% to >92% in mixed samples
- Unaltered gene expression, with no activation or changes in cellular response
- Achieve viable cell enrichment and maintain cellular heterogeneity

The LeviCell[®] platform delivers gentle touch-free, label-free cell preparation. The proprietary Levitation Technology[™] separates and enriches viable cells of interest from debris, dead cells, and other cell types while reducing hands-on time and handling steps by more than 80%. With the LeviCell systems streamlined, 3-step protocol, even the most fragile and sensitive cells can be enriched with high recovery rates and viability.

The LeviCell systems maintain starting population heterogeneity and minimize stress to maintain cell physiology, providing confidence in every precious sample prepared.



High Cell Viability and Recovery



Figure 1. Levitation enrichment increased viability from ~20% to >92% from a mixed starting sample of 200,000 live and dead Jurkat cells. Performance of the LeviCell system was validated by analyzing primary samples on a Sony® SH800S cell sorting instrument before and after enrichment with the LeviCell. Left panel: Ethanol-killed Jurkat cells were mixed with fresh live Jurkat cells to obtain a 20-25% viable mixed population. Dead cells were separated from the live cells and removed using the LeviCell 1.0 system. Two replicate experiments are shown. Right panel: A total of 200K cells were processed on the LeviCell 1.0 system to calculate yield recovered. Two replicate experiments demonstrate that average recovery is >85%, with individual experiments achieving 100% recovery.

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Biologically Relevant Results



Figure 2. Cellular expression, activation state and cellular response unaltered by levitation enrichment process. J774 cells (mouse, macrophages) were exposed to either IFN-y or IL-4, causing the up-regulation of iNOS (light bars) or Arg1 (dark bars), respectively. The results illustrate that compared to the untreated cells, IFN-y and IL-4 treatment was effective and processing with the LeviCell 1.0 system did not affect the expression of these genes or the cellular response to IFN-y or IL-4 treatment.

The LeviCell system's gentle cell enrichment enables the collection of notoriously delicate and sensitive primary cell types without altering the observed cell-type frequency of the original population. In this experiment, a mixed sample of peripheral blood mononuclear cells (PBMCs) were prepped, stained for visualization purposes, and levitated. Cellular ratios and overall sample heterogeneity of low frequency cell types like PBMCs were maintained through the LeviCell systems enrichment process.



Figure 3. Levitation enrichment separates mixed PBMC sample while maintaining original population heterogeneity. A mixed sample of PBMCs were prepared, and an aliquot was set aside. The remainder of the sample was levitated with the LeviCell 1.0 platform. All enriched samples and the reserved aliquot were then blocked and stained with anti-CD45 (PE), anti-CD3 (T-cells, FITC), anti-CD11b (monocytes, APC), and anti-CD19 (B-cells, PerCP-Cy5.5) for 1 hour on ice. Samples were washed with FACS buffer (0.5% BSA in PBS) and analyzed on a Sony® SH800S cell sorter. A similar amount of CD3+, CD19+, and CD11b+ cells within the CD45+ lymphocyte population were observed postenrichment with the LeviCell system compared to the pre-enriched sample.

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How It Works

Innovative label-free Levitation Technology facilitates complete debris and dead cell removal without affecting the original population representation or gene expression. In three simple steps, go from starting sample preparation to a purified, enriched sample containing cells of interest.



Single cell workflows, such as scRNA sequencing, often demand a minimum input of 50,000 cells, with recommended inputs going up to 1 million cells. With such daunting requirements, the chosen methodology for sample preparation must deliver not only viable cells, but high recovery rates to ensure the required cell counts are achieved. The LeviCell system's quick and gentle process translates into robust viability and high live-cell yield, while maintaining original sample heterogeneity, gene expression and activation states, making this cell separation solution the best choice for biologically relevant results from single cell analysis.

For more information, visit <u>https://levitasbio.com/single-cell-genomics/</u> or contact sales@levitasbio.com.

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