RESEARCH SNAPSHOT

Upstream Cold Tissue Dissociation Enhances Cell Enrichment to Deliver Superior Cell Viability

Overview

Isolating intact, viable cells from tissue samples can be challenging. Whether cells are from resections or biopsies, it is critical to maintain cell population representation ratios from the original sample while separating viable cells from dead cells and debris. Cells in solid tissues must first be dissociated from their neighbors by disrupting the cellular attachments to the extracellular matrix as well as the attachments between cells. This can be done through mechanical methods or proteolytic enzymes such as collagenase and trypsin, but each approach has the potential to damage cells or decrease yield. In addition, harsh dissociation and enrichment protocols can selectively damage more fragile cell types, changing the population distribution relative to the original population. Treatments can also stress the cells, potentially changing gene expression or altering cell surface markers. To minimize cellular damage and avoid perturbing gene expression profiles, researchers often perform tissue dissociation under lowered temperatures.

Regardless of the dissociation process used, the dissociated cells must be collected and removed from the resulting debris, and viable cells enriched by removing them from dead cells. Cells may be collected based on size or density by centrifugation, by binding to specific antibodies immobilized on beads or other matrix, or by using label-based sorting with fluorescently labeled antibodies bound to the cells of interest. These enrichment and debris removal processes typically involve multiple complex steps and expose sensitive cells to harsh physical and chemical conditions, leading to low viability and yield. In some instances with highly sensitive cell types within the tissue, these methods are completely incompatible. **Levitation Technology** presents an alternative to traditional enrichment protocols, providing high yields of viable cells while preserving cell distributions and characteristics for downstream applications and analysis.

KEY HIGHLIGHTS

- Elimination of complex steps and harsh physical/chemical conditions
- Superior viability from brain, lung, and liver samples:
  - Brain ~80%
  - Lung ~90%
  - Liver ~90%
- Seamless integration with levitation technology for further downstream enrichment and analysis

High Viabilities and Yields from Cold Dissociation

Cold tissue dissocation can better preserves cell health, which can be reflected by higher cell viability and possibly reduced early cell activation, early cell death or gene expression profile changes. The **LeviPrep™ Tissue Dissociation Kit**, which utilizes cold dissociation, was evaluated using murine tissues. To minimize handling steps and emulate a standard research workflow, fresh brain, liver, and lung tissues from PBS-perfused mice were purchased from The Jackson Laboratory. Each tissue was minced and dissociated on ice for 30 minutes per the tissue-specific enzymatic conditions. After cold digestion, initial viabilities ranged from an average of 75% for brain tissue, to 80% for liver tissue, and 86% for lung tissue. While these viabilities are relatively high, enrichment with the **LeviCell™ system** further improved their final viabilities (Figure 1).

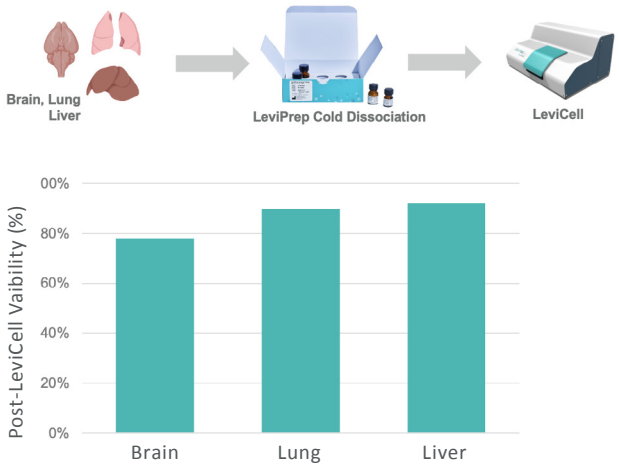

Figure 1. Viabilities of murine tissues after tissue dissociation and enrichment with the LeviCell system. *Dissociations were performed in duplicate. Bars shown represent the average cell viability.*

In Figure 2, cell yields after LeviPrep dissociation with different tissues types are shown. Brain averaged 4500 cells/mg of tissue, liver averaged 18,000 cells/mg of tissue, and lung averaged 25,000 cells/mg of tissue. Cold digestion may lead to lower cell yields, however, this protocol is optimized for preserving cell health and minimizing transcriptional changes during the dissociation workflow steps.

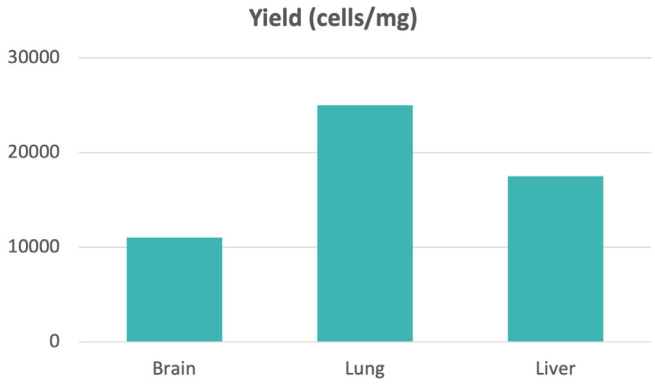

Figure 2. *Yields of cells after tissue dissociation.*

Cold Dissociation Preserves Transcriptional Profile

Tissue dissociation is often performed under warmer conditions, such as 37°C, where proteases can exhibit higher activity, potentially resulting in higher cell numbers. Higher temperatures can also lead to transcriptional changes since the transcriptional machinery remains active under these warmer conditions. In contrast, dissociations performed at colder temperatures can lead to a transcriptional profile that more accurately reflects the native state of the tissue (Denisenko et al., 2020, Genome Biology). Here, we demonstrate that high cell viability and high cell number can be maintained while operating under conditions that will preserve the native transcriptional profile. When post-dissociation viability is lower than desired, enrichment with the LeviCell system will improve sample viability, leading to improved downstream results as well.

Cold Dissociation and Levitation Technology for Highest Quality

Upstream cold tissue dissociation with the LeviPrep Tissue Dissociation Kit can preserve cellular health to deliver the most viable cells for your downstream tissue workflows. In combination with Levitation Technology, the LeviCell system efficiently removes dead cells and debris while producing high yields and viabilities from a variety of tissue and sample types, including fragile ones. Gene expression profiles are maintained while cell activation, manipulation and damage are minimized.

For more information, visit **levitasbio.com/leviprep**, or contact **sales@levitasbio.com**.