TECHNICAL NOTE

Lysis-Free Mouse Tissue RBC Removal and Viable Cell Enrichment in One Simultaneous Step

Overview

Conducting research with mouse tissues expands the fundamental understanding of biology and specific pathologies. Cell suspensions from dissociated tissues are critical for translational research and drug discovery. However, debris, dead or dying cells, and tissue contaminants such as red blood cells (RBC) or structural proteins can have a negative impact on subsequent downstream experiments. In order to obtain high-quality data, removal of these contaminants is required, which can take multiple labor-intensive steps.

In particular, RBC contamination in dissociated tissues introduces errors to the measured concentration and viability of the cells of interest. This can be detrimental to downstream applications such as flow cytometry, functional assays, and single-cell RNA sequencing. If the cell number or concentration cannot be confidently established, inaccuracies in the cells loaded per reaction or contribution of RNA noise can occur. This inadvertently creates poor scRNA-Seq QC metrics or forces an increase in the amount of sequencing needed to detect cell populations of interest. For specific research areas like cell therapy, RBCs in bone marrow mononuclear cell samples can affect viability and mitochondrial function, impairing some key functionalities (Assmus et al. 2010).

Although RBCs can be removed during data analysis using gene expression markers, the most common method scientists rely on is a lysis buffer protocol. Typically, an ammonium chloride-based solution is added to the whole sample, creating a hypotonic environment that preferentially lyses non-nucleated RBCs. This process can result in premature apoptosis of sensitive cell types, and cells of interest. It is recommended that cell viability be greater than 90% for scRNA-seq and samples with less than 70% viability should undergo some dead cell removal step. This ensures the final sample is composed of only healthy, viable cells and any dying or dead cells are removed which can interfere with downstream applications.

KEY HIGHLIGHTS

- Gentle and efficient RBC depletion from mouse tissue
- Proven for a wide range of tissues and starting cell numbers
- Simultaneous RBC depletion and viable cell enrichment

The LeviSelect™ Mouse Tissue RBC Depletion Kit selectively depletes RBCs from mouse tissue, avoiding lysis while simultaneously enriching viable cells. In a few short steps, RBCs in the sample are labeled for depletion. Once the sample has been loaded into the cartridge on the LeviCell® system, the unwanted RBCs are retained against the bottom and sides of the cartridge due to the magnetic forces. Users achieve simultaneous depletion of RBCs and enrichment of viable cells that can be used for downstream processing after collection.

Low viability and poor-quality samples with as few as 20,000 cells would be considered unrecoverable with other RBC removal methods. RBC lysis, for example, would require multiple washes, and cell loss would be inevitable. The following technical note outlines the performance of the LeviSelect Mouse Tissue RBC Depletion Kit across different tissues that range in starting viability and cell input.

RBC Depletion Performance Across Different Numbers of Cells and Tissues

The LeviSelect Mouse Tissue RBC Depletion Kit utilizes a two-step protocol in which a single-cell suspension is incubated first with an RBC antibody cocktail, followed by an incubation with paramagnetic nanospheres. Afterward, the Levitation Agent is added to a final concentration of 150 mM, and 220 µL of the mixture and

is loaded onto the LeviCell platform without washing. The fixed magnets within the LeviCell systems attract the nanospheres-bound RBCs to the walls of the cartridge. At the same time, the unbound cells levitate within the paramagnetic levitation buffer, separating viable cells from dead cells. When the unbound cells have reached equilibrium, the viable cells can be collected into the outlet well and are ready for downstream processing. An unperturbed viable cell suspension, free of RBC contamination, can be achieved in less than an hour.

There are multiple methods for measuring the depletion of RBCs and the performance of the LeviSelect Mouse Tissue RBC Depletion Kit (such as flow cytometry, microscopy, quantification of hemoglobin, and scRNA-seq). Flow cytometry can assess the depletion performance by detecting specific RBC-related proteins. In Figure 1 the detection of Glycophorin A protein, the major transmembrane sialoglycoprotein of red blood cells, is plotted without (Fig1A) and with depletion (Fig1B) for a spleen cell suspension. The 2D dot plots show the decrease in the RBC population from approximately 50% to 0.1 % after using the RBC depletion kit.

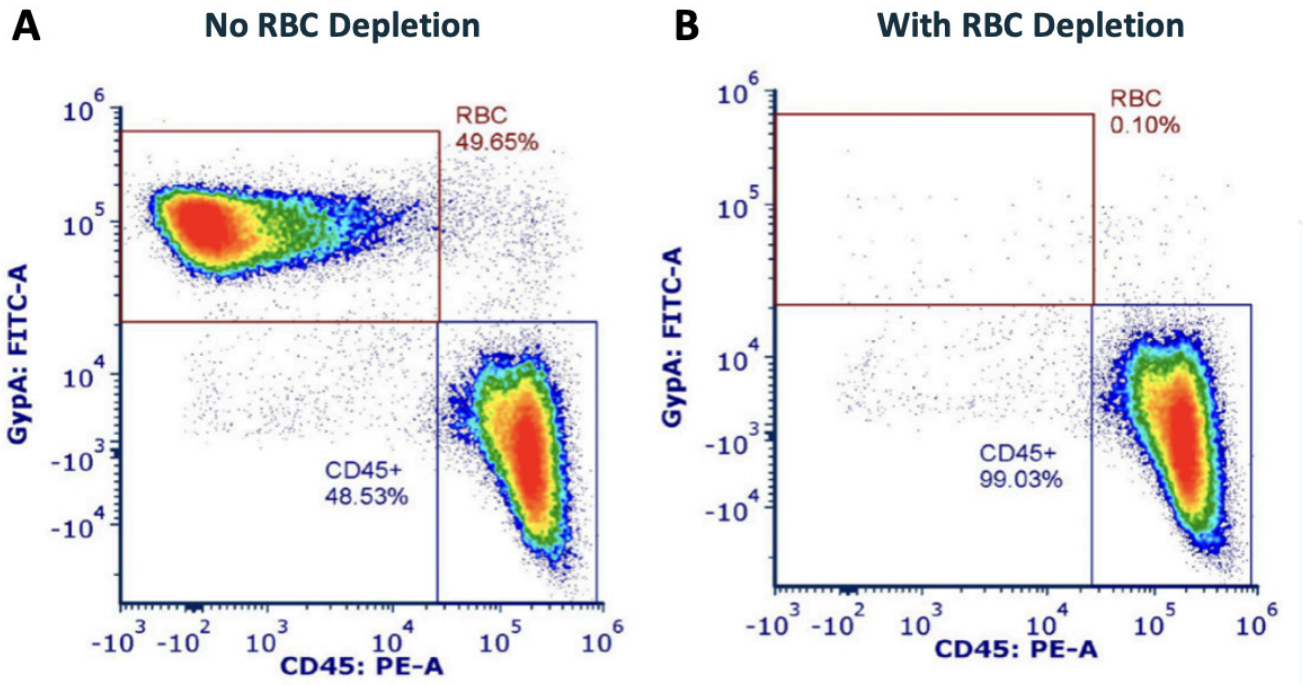

Figure 1. Detection of RBCs before and after using the LeviSelect Mouse Tissue RBC Depletion Kit. *Mouse splenocytes were subjected to RBC depletion and then stained for glycophorin A-FITC (RBC marker), CD45-PE (immune cell marker) and propidium iodide (PI, dead cell marker). Stained cells were analyzed by flow cytometry, gating on singlets and then live cells using PI. A) A non-depletion control (that was levitated with nanospheres but not RBC-targeting antibody cocktail) is shown for comparison to the (B) RBC-depleted sample.*

During levitation on the LeviCell system, the sample can be immediately assessed for the depletion of RBCs. To demonstrate this, a parallel mock RBC depletion (like a standard viable cell enrichment) of mouse lung tissue cells (top panel) was run on the same LeviCell EOS-4 cartridge as a RBC-depleted lung sample (bottom panel). In the top panel, the thick dark band of contaminating RBCs can be seen overlapping at the same height where viable cells levitate while in the bottom panel, the depleted RBC population is retained on the edges of the separation channel leaving only the levitated cells of interest visible in the top half of the channel. The ability to visualize cells during the sample processing step gives the researcher real-time feedback on the sample quality.

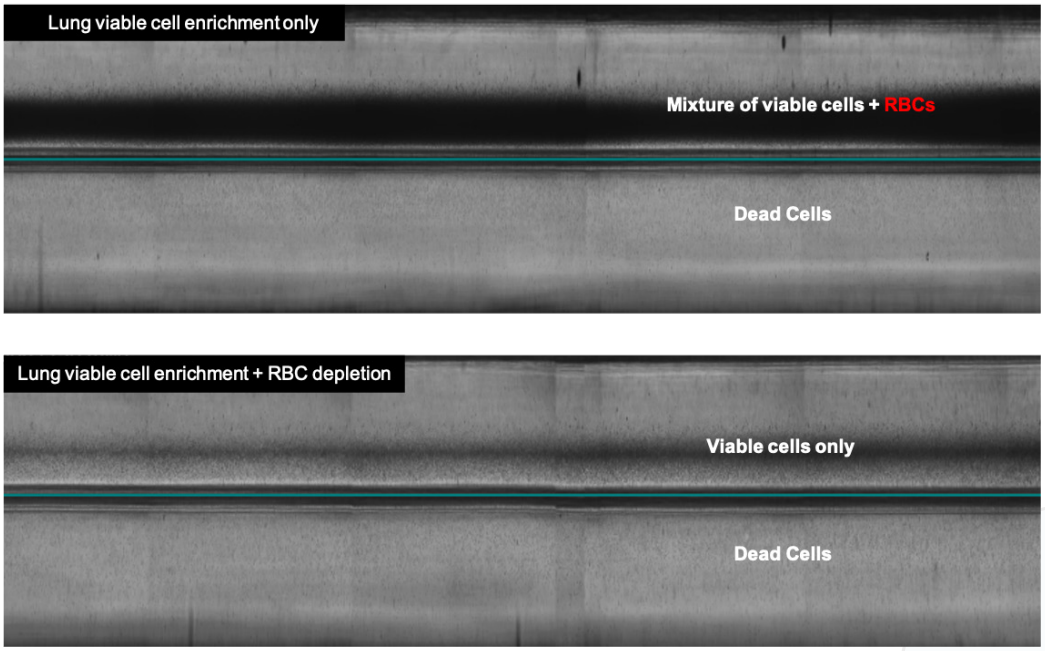

Figure 2.RBC depletion causes a distinctive thinning of the viable cell band when compared to the non-depletion control. *0.8 million total nucleated lung cells (83% viable) and 3.3 million RBCs loaded are shown after levitation without depletion* ***(top panel)*** *and with depletion of RBCs* ***(bottom panel).*** *In both separation channels, viable lung cells levitate above the split line (teal line shown in each of the two separation channels); dead cells and debris are distributed below the split line. The user interface allows for a qualitative sample assessment and comparison across the two samples loaded in the cartridge, allowing for a prompt visualization of the remaining RBCs in the non-depletion control sample.*

The kit's success can also be evaluated immediately by microscopy without requiring the long staining protocol and expensive fluorescently-labeled antibodies that are required for flow cytometry. The quantification of RBCs before and after depletion using the Mouse Tissue RBC Depletion Kit can be assessed using brightfield microscopy to count all cells, in combination with nucleated cell stains like Acridine Orange and Propidium Iodide (AOPI), which specifically count all nucleated cells. As RBCs don't contain nuclei, the non-nucleated events can be labeled as RBCs.

Using this quick and efficient methodology, in Figure 3, the depletion rates and viability improvement for 102 depleted mouse spleen and lung samples are shown across varying amounts of nucleated cells and RBCs loaded. In Figure 3a, cell viability improvements pre-LeviCell (grey) and post-LeviCell (teal) were equally effective at all input ranges, even as low as 90,000 starting cell number. In Figure 3b, independent of the amount of RBCs loaded, the depletion rates were close to 100% for all samples. This confirms that the LeviCell platform can remove the majority of RBCs.

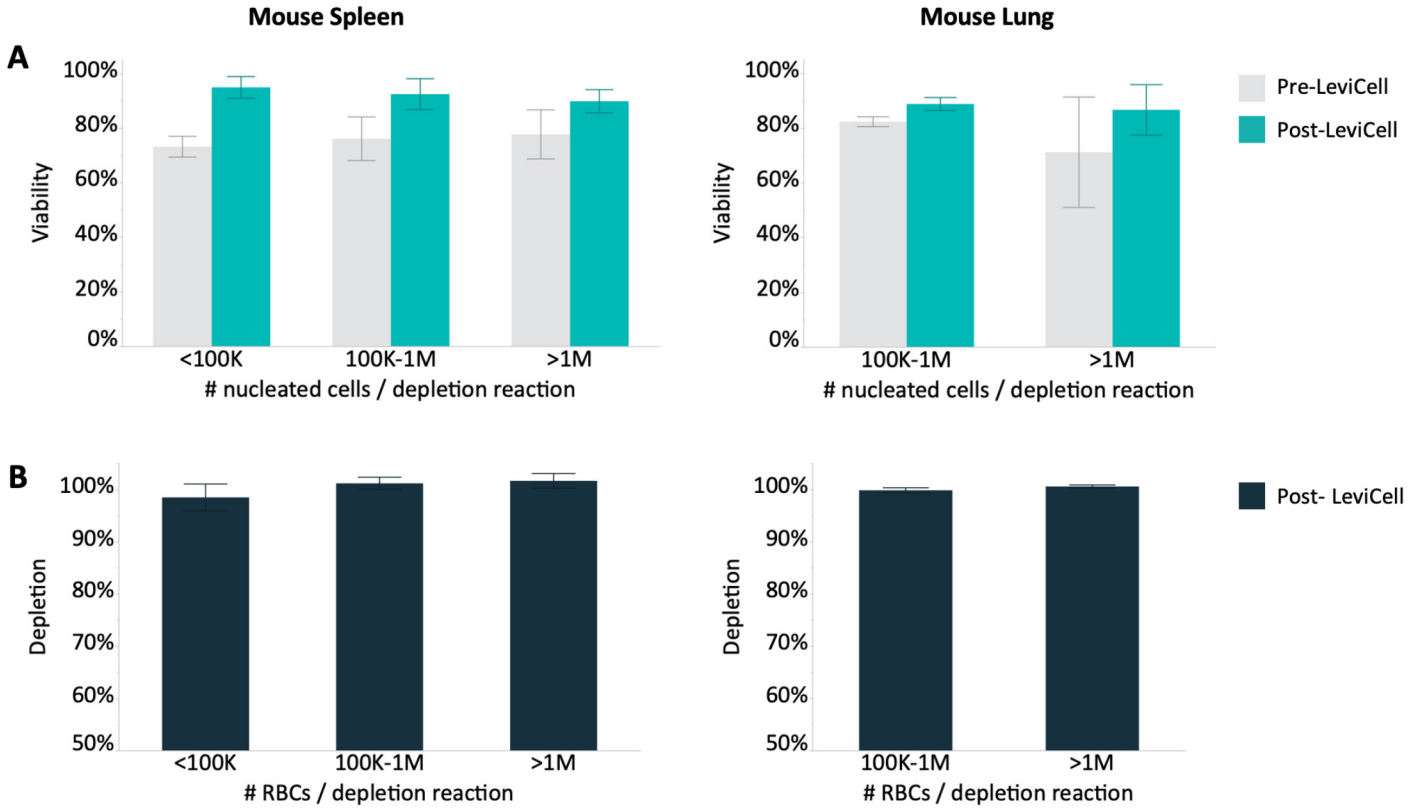

Figure 3. Mouse spleen and lung viability and RBC depletion on the LeviCell system across a wide range of input cell numbers. All ranges show consistent improved viability of nucleated cells as well as consistent depletion of RBCs. A) *Viabilities of nucleated cells were determined by nuclei fluorescence (AOPI) from lung samples (n=15) and spleen samples (n=87) before and after loading into the LeviCell platform, shown in grey and teal respectively.* ***B)*** *The average depletion rate across different ranges of loaded RBCs was calculated using the number of RBCs before and after the depletion. These numbers were determined by capturing the brightfield and nuclei fluorescence (by Acridine Orange and Propidium Iodide) from each sample before and after loading the LeviCell platform.*

In addition to lung and spleen mouse tissue, the kit exhibits high rates of RBC depletion across other mouse tissues like pancreas, brain and liver while improving their starting viability significantly. While lung and spleen cell suspensions can easily be quantified, many other tissues present more confounding amounts of debris after dissociation, making their evaluation by brightfield microscopy challenging and potentially inaccurate. Moreover, if studying disease models and their tissues, additional necrosis or debris can create more challenges. Table 1 summarizes the different tissues tested with the LeviSelect Mouse Tissue RBC Depletion Kit. The methodology used to assess the depletion rate was based on the amount of hemoglobin present before and after the RBC depletion. Viability was measured by AOPI staining to ensure the counting of nucleated cells.

Mouse Tissue	Depletion Rate % (mean ± STDEV)	Viability before	Viability after (mean ± STDEV)
Pancreas	97.7 ± 0.0	39.1%	70.2% ± 1.8
Brain	98.1 ± 0.2	65.3%	89.0% ± 10.5
Liver	99.4 ± 0.1	69.0%	89.0% ± 10.6
Spleen	99.8 ± 0.0	69.1%	94.9% ± 3.2
Lung	99.0 ± 0.1	80.7%	90.6% ± 1.2

Table 1. High depletion rates and increased viability seen across multiple mouse tissues after LeviSelect Mouse Tissue RBC Depletion on the LeviCell system. *Each depletion contained 100,000 live cells and different amounts of RBCs depending on the tissue (n=3). The number of RBCs before and after were assayed using the hemoglobin colorimetric detection kit (Arbor Assays) measured on a plate reader.*

Conclusions

Every workflow step can introduce variation, cell loss, unintentional cell death, or changes in gene expression. Using Levitation Technology and the LeviCell platform, researchers can now streamline their workflow when working with mouse tissue by simultaneously removing RBC contamination from tissue and enriching for viable cells of interest. By avoiding RBC lysis, researchers can prevent bystander cell death, reduce the number of workflow steps, and minimize any chance of cell loss from washing or centrifugation. The LeviSelect Mouse Tissue RBC Depletion Kit and LeviCell system can accelerate sample processing workflows and help uncover true biological cell profiles. This novel approach will help to preserve cell integrity and native gene expression while avoiding costly trade-offs that can affect downstream analysis.

References

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