

LeviSelect Mouse Tissue RBC Depletion Kit (10 rxn)

PRODUCT DATA SHEET

Catalog# 1004112

RUO: For Research Use Only. Not for use in diagnostic procedures.



Description

The LeviSelect™ Mouse Tissue RBC Depletion Kit has been designed to deplete up to 5 million mature erythrocyte and erythroid precursor cells per reaction after a mechanical or enzymatic tissue dissociation while simultaneously enriching for live cells. The depleted tissue red blood cells (RBCs) remain bound to the cartridge during a run on the LeviCell® systems. At the same time, the remaining viable, untouched cells will be separated from dead cells in the suspension and then collected in the top fraction output of the cartridge. The bottom fraction will consist primarily

of dead cells, as the depleted erythroid cells will be immobilized inside the cartridge.

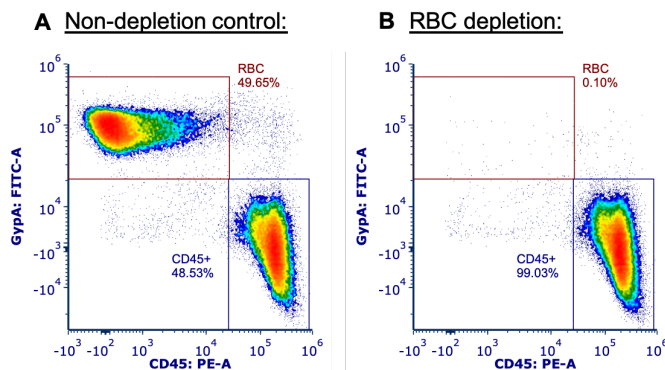
RBCs are identified and bound using a biotinylated mouse RBC antibody cocktail. Streptavidin magnetic nanospheres then bind these biotin-labeled RBCs. When loaded into the LeviCell systems cartridge placed within the magnetic field in the LeviCell platforms, the nanosphere-coated cells are depleted from the suspension, leaving the cells of interest in suspension.

Data Example

Table 1. Depletion rate after LeviSelect Mouse Tissue RBC Depletion Kit across different mouse tissues. 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 measured using the Hemoglobin Colorimetric Detection Kit (Arbor Assays) using a plate reader.

| Mouse Tissue | Depletion Rate % (mean ± STDEV) |
|--------------|---------------------------------|
| Pancreas | 97.74 ± 0.03 |
| Brain | 98.10 ± 0.17 |
| Liver | 99.43 ± 0.06 |
| Spleen | 99.82 ± 0.01 |
| Lung | 99.00 ± 0.05 |

Figure Legend. Detection of RBC 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 then analyzed by flow cytometry, gating first on singlets and then live cells excluding PI. A) A non-depletion control (that was levitated with nanospheres but not RBC-targeting antibody cocktail) is shown for comparison to the RBC depleted sample (B).



Kit Components

| Component | Tube PN | Quantity | Storage Conditions | Prep for use |
|--|---------|-----------------|----------------------------------|-----------------|
| LeviSelect SAV Nanospheres, Tube | 6000069 | 1 x 100 μ L | Store at 2-8°C. Do not freeze | Maintain on ice |
| 1X LeviSelect Buffer, Tube | 6000027 | 2 x 1.8 mL | Store at 2-8°C. Do not freeze | Maintain at RT |
| LeviSelect Mouse RBC Ab Cocktail, tube | 6000097 | 1 x 10 μ L | Store at 2-8°C. Do not freeze | Maintain on ice |

Required Buffers

| Buffer | Function |
|-----------------------|--|
| 1X LeviSelect Buffer | Buffer used to resuspend cell mixtures in preparation for cell enrichment reactions. Provided with the LeviSelect kit. |
| Levitation Agent (1M) | Concentrated stock of levitation agent |
| Levitation Buffer | Buffer made by combining 1X LeviSelect Buffer with Levitation Agent to make a working Levitation Agent concentration to be added to cells prior to loading on the LeviCell |

Required Tools and Consumables

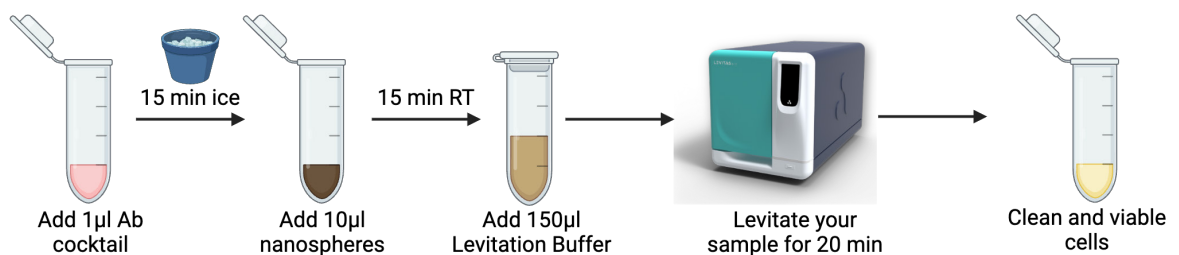
LeviCell 1.0 System; LevitasBio PN 1000001
 Or LeviCell EOS System PN 1000020
 LeviCell S2.3 Cartridge; LevitasBio PN 1002010
 or LeviCell S2.3-IR Cartridge; PN1002012
 LeviCell EOS-4 Cartridge; LevitasBio PN 1002101

Levitation Agent; LevitasBio PN 1003001
 1.5 -2.0 mL low-bind microcentrifuge tubes
 0.1-2 μ L, 2-20 μ L, and 200-1000 μ L pipettes and tips
 Cell culture grade water

- If using samples for flow cytometry: Do not label them with fluorescent antibodies until the depletion is performed.
- Account for an extra tube with the same number of cells if measurement of the total cell number and viability is required for the pre-depleted sample (e.g. the input).

Before Getting Started

Workflow



LeviSelect Mouse Tissue RBC Depletion Kit Protocol

1. Prepare a cell suspension from the tissue of interest.
2. Count cell suspension for both viability and cell concentration.
3. Aliquot between 2×10^4 to 5×10^6 nucleated live cells ($\sim 10 \mu\text{m}$ cell size), the upper limit may decrease for larger sized cells, up to 5×10^6 RBC contamination or 1:1 cell:RBC from the prepared cell suspension into a new 1.5 mL or 2 mL low-bind tube. If using more cells, use more tubes aliquoting in each of them no more than 5×10^6 live nucleated cells.
4. Centrifuge the cells at 300 RCF for 5 minutes. Remove and discard the supernatant.
5. Resuspend cell pellet in 110 μL of 1X LeviSelect Buffer.
6. Pipet mix the tube containing the biotinylated antibody and add 1 μL to the resuspended cells. Pipette mix with $>80 \mu\text{L}$ 10 times.
7. Incubate cell suspension with the biotinylated antibody for 15 minutes on ice.
8. Pipet mix the tube containing the LeviSelect SAV Nanospheres and add 10 μL of LeviSelect SAV Nanospheres to the resuspended cells. Pipette mix with $>80 \mu\text{L}$ 10 times. Total volume is now 120 μL . DO NOT add nanospheres to input tube for counting.
9. Incubate cell suspension with the LeviSelect SAV Nanospheres for 15 minutes at room temperature.

! NOTE: The LeviCell cartridge works optimally with samples at ambient temperature.

| Reagent Volume (μL) | For 1 depletion | For 1 depletion + Input Control | For 4 depletions | For 4 depletions + Input Control | Storage Conditions |
|----------------------------------|-----------------|---------------------------------|------------------|----------------------------------|---------------------------|
| 1X LeviSelect Buffer | 131 | 263 | 526 | 657 | Store at room temperature |
| Levitation Agent | 49 | 97 | 194 | 243 | Store at room temperature |
| Total | 180 | 360 | 720 | 900 | Store at room temperature |

10. Add 150 μL of prepared Levitation Buffer to the 2 mL tube. Total volume is now 270 μL .
11. Pipette mix with the same pipet tip 10 times. The magnetic nanospheres should be uniformly dispersed throughout the solution.
12. Set up the LeviCell cartridge on the instrument following the instructions on the Experiment Manager User Interface, selecting the “standard” option when using the LeviCell and the “medium” option when using the EOS.
13. With a P1000 pipet set to 220 μL , pipet up and down 10X to mix thoroughly (avoid bubble formation) and load 220 μL of cell suspension into the inlet well of the cartridge. The pipette tip should be placed near the backside of the well, slightly above the entrance to the flow channel.

! NOTE: Avoid introducing bubbles into the inlet well by not depressing the pipette plunger past its initial stop.
14. Start the LeviCell run.
15. Follow all instructions on the LeviCell up to and including the sample retrieval step. The split line recommended is 0. Your desired cells will be collected in the top fraction.