LeviSelect Mouse Tissue RBC Depletion Kit (10 rxn)

PRODUCT DATA SHEET

Catalog# 1004110

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.

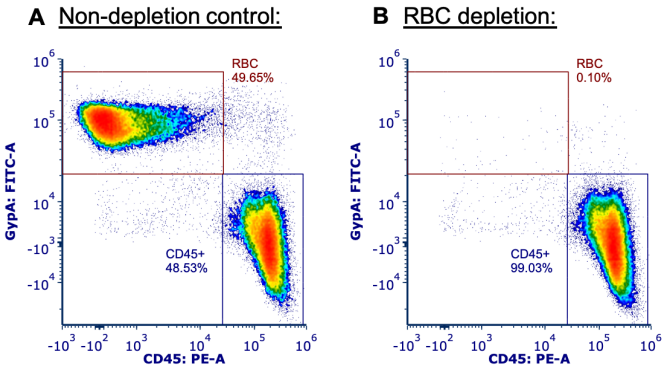
RBCs are identified and bound using a biotinylated mouse RBC antibody cocktail. Streptavidin magnetic nanospheres then bind these biotin-labeled RBCs. When loaded into the LeviCell systems cartridge placed within the magnetic field in the LeviCell platforms, the nanosphere-coated cells are depleted from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

LeviSelect Mouse Tissue RBC Depletion Data

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

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 µL	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 µL	Store at 2-8°C. Do not freeze	Maintain on ice

Required Buffers

Buffer	Function
1X LeviSelect Buffer[1]	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

[1]LeviSelect buffer contains 2mM EDTA which should be washed out after levitation and before proceeding to downstream applications involving sequencing analysis.

Required Tools and Consumables

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

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

Before Getting Started

- If using samples for flow cytometry: do not label them with fluorescent antibodies until RBC depletion has been completed.
- If working with samples that contain cells expressing significant amounts of Fc receptors (e.g. microglia), it is advisable to pre-incubate these cell samples with an Fc-blocking antibody cocktail prior to adding the RBC-targeting antibody cocktail.

LeviSelect Mouse Tissue RBC Depletion Kit Protocol

Workflow

Protocol

1. Prepare a cell suspension from the tissue of interest.
2. Count cell suspension for both viability and cell concentration.
3. Aliquot between 2x10^4 to 5x10^6 nucleated live cells (~10 µm cell size the upper limit may decrease for larger-sized cells), with up to 5x10^6 RBC contamination 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 5x10^6 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 µL 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 µL 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.

10. Prepare Levitation Buffer containing 150mM Levitation Agent at room temperature (all the calculations include 20% overage). For other combinations scale these volumes accordingly.

Reagent Volume (µL)	1 depletion rxn	1 depletion rxn + 1 input control	4 depletions rxns	4 depletions rxns + 1 input control
1X LeviSelect Buffer	131	263	526	657
Levitation Agent	49	97	194	243
Total	180	360	720	900

11. Add 150 µL of prepared Levitation Buffer to the 2 mL tube. Total volume is now 270 µL.
12. Pipette mix with the same pipet tip 10 times. The magnetic nanospheres should be uniformly dispersed throughout the solution.
13. 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.
14. 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.

15. Start the LeviCell run.
16. Follow all instructions on the LeviCell up to and including the sample retrieval step. The split line recommended is 0. Desired cells will be collected in the top fraction.