LeviSelect Human Tissue RBC Depletion Kit (10 rxn)

PRODUCT DATA SHEET

Catalog# 1004112

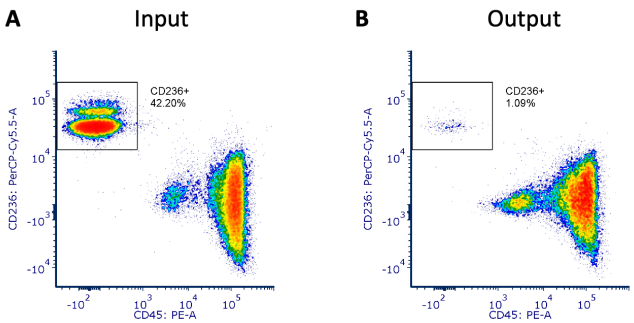
RUO: For Research Use Only. Not for use in diagnostic procedures.

Description

The LeviSelect™ Human 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 red blood cells (RBCs) remain bound to the cartridge during a run on the LeviCell® systems, while viable, untouched cells of interest are enriched and collected in the top fraction output of the cartridge. The bottom fraction will consist primarily of dead/dying cells and debris, whereas the depleted erythroid cells remain immobilized inside the cartridge during the collection step. RBCs are identified and bound using a biotinylated human RBC antibody cocktail. Streptavidin magnetic nanospheres then bind these biotin-labeled RBCs. When loaded into the LeviCell system's 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.

Human Tissue RBC Depletion Data

Detection of RBC after using the LeviSelect Human Tissue RBC Depletion Kit. *A human PBMC sample contaminated with RBC was subjected to RBC depletion and both the input (A) and output (B) top fractions were stained for flow cytometry analysis using anti-CD236 (RBC marker), CD45 (immune cell marker), and PI (dead cell marker). Representative flow plots were obtained by first gating on singlets, then live cells excluding PI and finally plotting CD236 vs. CD45 staining.*



Kit Components

Component	Tube PN	Quantity	Storage Conditions	Prep for use
LeviSelect SAV Nanospheres, Tube	6000099	1 x 10 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 human RBC Ab Cocktail, tube	6000098	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 Instrument; 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
(if using the LeviCell EOS)

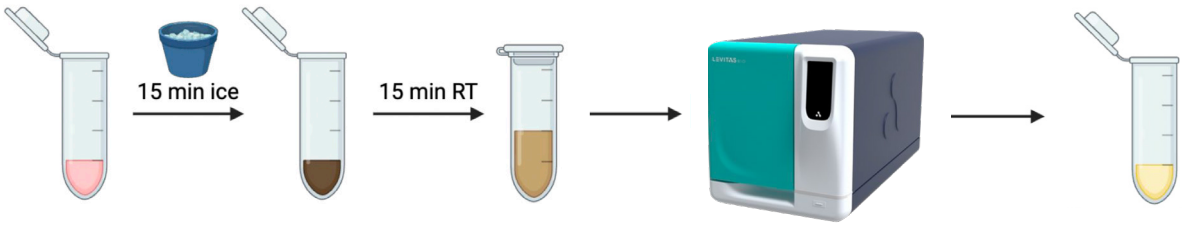
Levitation Agent; LevitasBio PN 1003001
1.5 -2.0 mL low-bind microcentrifuge tubes
0.1-2 µL, 2-20 µL, and 200-1000 µL pipettes and tips
Cell culture-grade water

Before Getting Started

- If using samples for flow cytometry: do not label them with fluorescent antibodies until the depletion has been performed.
- 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.
- Account for an extra tube with the same number of cells if the total cell number and viability measurement are required for the pre-depleted sample (e.g., the input).
- When working with less than 250,000 nucleated cells, the yield of monocytes can be improved by scaling down the nanospheres. The recommendation is to dilute the nanospheres 1:10 and use 1 µL of that dilution.

LeviSelect Human 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 2x 10^4 to 5x 10^6 nucleated live cells (~10 µm cell size, the upper limit may decrease for larger sized cells) 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 5x 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 120 µL of 1X LeviSelect Buffer.
6. Briefly spin down the tube containing the biotinylated antibody, pipet mix and then 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 1 µL of LeviSelect SAV Nanospheres to the resuspended cells. Pipette mix with >80 µL 10 times. DO NOT add nanospheres to the 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 resuspended cells. Pipette mix with the same pipet tip 10 times. The magnetic beads 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. Desired cells will be collected in the top fraction.

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