LeviSelect Mouse CD45 Depletion Kit (10 rxns)

Part Number #1004003
For Research Use Only. Not for use in diagnostic procedures.

Description

The LeviSelect™ Mouse CD45 Depletion Kit is designed to deplete mouse CD45+ cells from a mouse cell suspension during live cell enrichment on a LeviCell™ system. The CD45+ cells will remain bound to the LeviCell cartridge, while the viable CD45- population is separated from dead cells in the suspension and then collected in the top outlet well of the cartridge. The bottom outlet well will consists primarily of dead CD45- cells as the CD45+ cells will be immobilized inside the cartridge.

The mouse CD45+ cells are bound by magnetic nanospheres conjugated with an anti-mouse CD45 antibody. When loaded into the LeviCell cartridge placed within the magnetic field in the LeviCell instrument, the nanosphere-coated CD45+ cells are selectively depleted from the suspension.

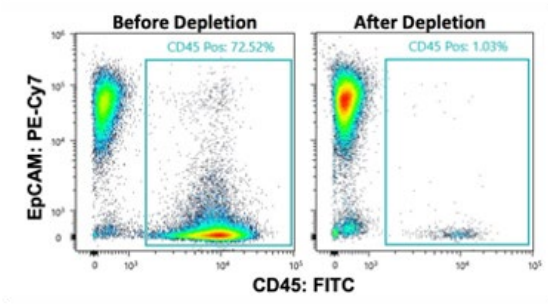

Figure 1. The LeviSelect Mouse CD45 Depletion Kit highly enriches CD45- cells. *Heterogeneous mixture of BALB/c splenocytes (CD45+) and murine HC11 mammary epithelial cells (CD45-) were analyzed using flow cytometry . LeviSelect CD45 depletion resulted in 99% depletion of CD45+ cells, resulting in a population highly enriched for CD45- cells. All live cells used for analysis.*

Kit Components

Component	Tube PN	Quantity	Storage Conditions	Shelf Life
Anti-mouse CD45+ Nanospheres	6000035	1 x 25 µL	Store at 2-8°C. Do not freeze	Stable until expiration date on label
1X LeviSelect Depletion Buffer	6000027	2 x 1.8 mL	Store at 2-8°C. Do not freeze	Stable until expiration date on label

Additional Tools and Consumables Required

- LeviCell-1.0 Instrument (PN 1000001)
- LeviCell S2.3 Cartridge (PN 1002010) OR LeviCell S2.3-IR cartridge (PN 1002012)*
- Levitation Agent (PN 1003001)
- 1.5 -2.0 mL low bind microcentrifuge tubes
- P2 or P10, P20 and P1000µL pipettes and tips

* LeviCell S2.1 and S2.1-IR cartridges are also compatible.

Additional Resources

- 90-00204 LeviCell Instrument User Guide, including Live Cell Enrichment Protocol
- 90-00213 Quick Reference Guide - Live Cell Enrichment

LeviSelect Mouse CD45 Depletion Protocol

Prepare Levitation Buffer

Prepare at room temperature. When prepared as indicated below, the Levitation Agent will have a final concentration of 150mM when added to the labeled cells.

Reagent Volume	Volume for 1 sample (µL)**	Volume for 4 samples (µL)**
1X LeviSelect Depletion Buffer	10.9	43.7
Levitation Agent	46.6	186.3
Total	57.5	230

** Volumes account for 15% overage

LeviCell Run with CD45+ Cell Depletion

1. Prepare a cell suspension from tissue/cells of interest.
2. Count cell suspension for both viability and cell concentration. Aliquot 1.25 x 10^6 live cells from the prepared cell suspension into a new 2 mL low bind tube. Centrifuge the cells at 300xg for 5 minutes. Remove and discard the supernatant. Resuspend cell pellet in 218 µL of 1X LeviSelect Depletion Buffer (216 µL if prioritizing purity).

   Note: Antibody labelling of the sample prior to incubation with the nanospheres may disrupt binding of the cells to the nanospheres and inhibit depletion. It is recommended that all antibody labelling be performed after depletion.
3. Vortex the tube containing the anti-muCD45+ nanospheres at maximum speed for 5 pulses to thoroughly resuspend the solution and nanospheres.
4. Add 2 µL of anti-muCD45+ nanospheres to the resuspended cells. Pipette mix with >100 µL 10 times. Total volume will be 220 µL.

   Note: The volume of anti-mouse CD45+ nanospheres may be adjusted depending on the priorities of the experiment. If higher purity is preferred, the volume of nanospheres can be increased to 4 µL. When doing so, the cell pellet from step 2 should be resuspended in 216 µL of 1X LeviSelect Depletion Buffer. This may result in lower yield of CD45- cells.
5. Incubate cell suspension with the anti-muCD45+ nanospheres for 5 minutes at room temperature. Do not incubate on ice.

   Note: The LeviCell cartridge works optimally with samples at ambient temperature. Cold samples can cause thermal movement of the liquid in the cartridge that can interfere with levitation equilibrium.
6. Add 50 µL of prepared Levitation Buffer to the 2 mL tube. Total volume will be 270 µL.
7. Set up the LeviCell cartridge on the instrument following the instructions on the Experiment Manager User Interface, selecting the “standard” option.
8. With a P1000 pipet set to 220 µL, pipet up and down 5X to mix thoroughly, avoiding 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: Prevent the introduction of bubbles into the inlet well by not depressing the pipette plunger past the initial stop.
9. Start the LeviCell run.