LeviSelect Human CD45 Depletion Kit (10 rxn)

PRODUCT DATA SHEET

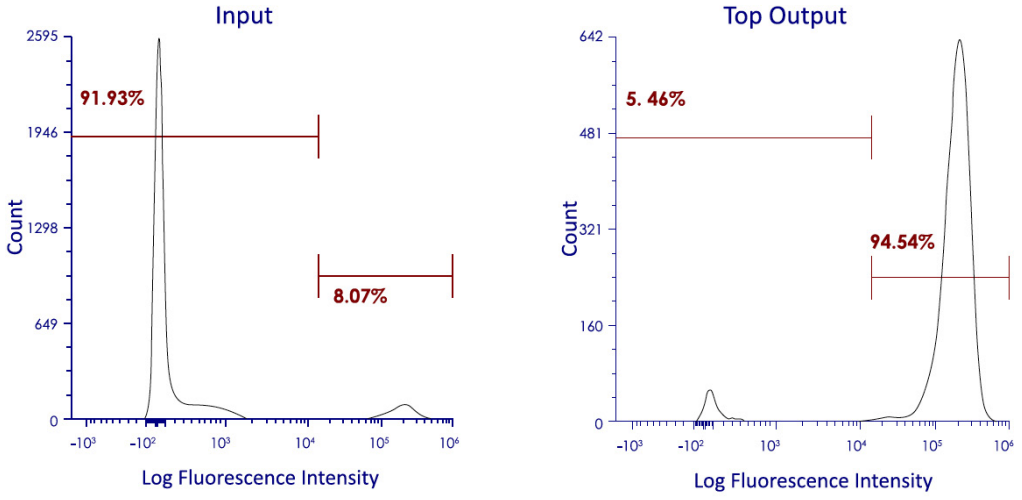
Part Number #1004001
For Research Use Only. Not for use in diagnostic procedures.

Description

The LeviSelect™ Human CD45 Depletion Kit is designed to deplete human CD45+ cells from a cell suspension during live cell enrichment on a LeviCell™ instrument and cartridge. The CD45+ cells will remain bound to the LeviCell cartridge consumable, while the CD45- population is separated from dead cells in the suspension and then collected in the top output well of the cartridge.

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

Data Example



NOTE: *EpCAM is an antibody against an epithelial cell surface antigen expressed on H358 cells.*

Figure 1. CD45+ targeted depletion is confirmed through comparison of input and output cell counts. *A mixed cell suspension targeted at 10% CD45- H358 cells and 90% CD45+ human PBMCs was prepared. 1M total cells were run on the LeviCell after 5 min incubation with 20 µL LeviSelect CD45 nanospheres. Cells were stained with EpCAM PE-Cy7. Dead cells were excluded by 7-AAD.*

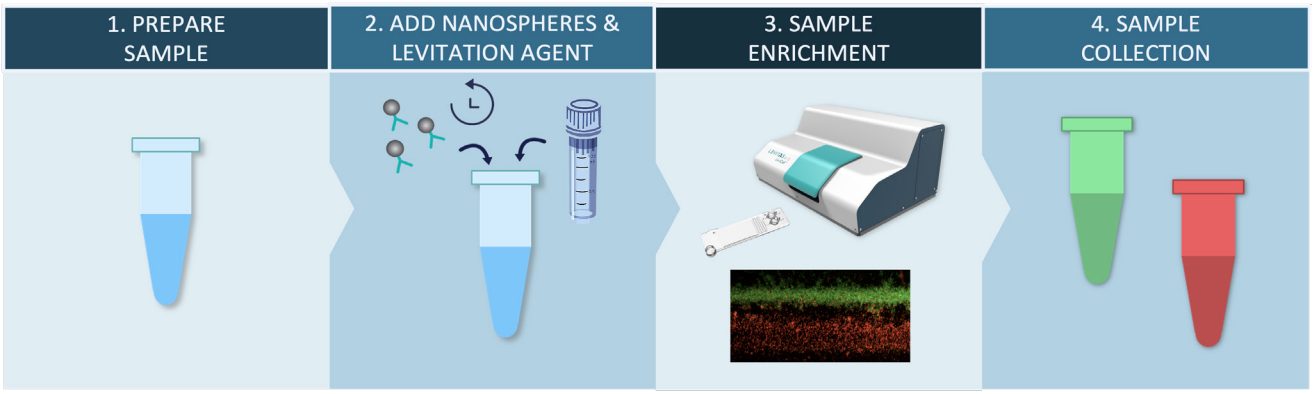
Kit Components

Component	Tube PN	Quantity	Storage Conditions	Shelf Life
Anti-human CD45+ Nanospheres	6000029	1 x 125 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
- 2-20 µL and 200-1000 µL pipettes and tips

LeviSelect Workflow



Additional Resources

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

LeviSelect Human CD45 Depletion Kit Protocol

Prepare Levitation Buffer

For preparation of final Levitation Buffer with concentration of 150mM Levitation Agent.

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

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 tube 2 mL low bind tube. Centrifuge the cells at 300xg for 3 minutes. Remove and discard the supernatant. Resuspend cell pellet in 200 µL of 1X LeviSelect Depletion Buffer.
3. Vortex the tube containing the anti-huCD45+ nanospheres at maximum speed for 5 pulses to thoroughly resuspend the solution and nanospheres.
4. Add 20 µL of anti-huCD45+ nanospheres to a new 2 mL low bind tube.
5. Add 200 µL of the cell suspension to the 2 mL tube containing the CD45 depletion nanospheres and mix well by pipetting up and down 10X.
6. Incubate cell suspension with the anti-huCD45+ 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.

7. Add 50 µL of prepared Levitation Buffer to the 2 mL tube. Total volume is now 270 µL.
8. Set up the LeviCell cartridge on the instrument following the instructions on the Experiment Manager User Interface, selecting the “standard” option.
9. With a P1000 pipet set to 220 µL, pipet up and down 5X 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.

10. Start the LeviCell run.