LeviSelect Human CD8 T Cell Enrichment Kit (10 rxn)

PRODUCT DATA SHEET

Part Number #1004009
For Research Use Only. Not for use in diagnostic procedures.

Description

The **LeviSelect™ Human CD8 T Cell Enrichment Kit** is designed to enrich human CD8+ T cells from a peripheral blood mononuclear cell (PBMC) sample during viable cell enrichment on a **LeviCell™ 1.0 instrument** and cartridge. All non-CD8+ T cells will remain bound to the LeviCell cartridge consumable, while the untouched viable CD8+ T cell population is 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 CD8+ T cells as the depleted cells will be immobilized inside the cartridge.

The non-CD8+ T cells are bound by a biotinylated cocktail of antibodies against the remaining cell populations within the PBMC sample, and the antibodies are then bound by magnetic nanospheres. When loaded into the LeviCell cartridge placed within the magnetic field in the LeviCell instrument, the nanosphere-coated cells are depleted from the suspension, leaving the CD8+ T cells in suspension.

Kit Components

Store all kit components at 2-8˚C. Do not freeze. Components are stable through stated expiration date.

LeviSelect hCD8 SAV Nanospheres (1 tube)
PN 6000042, 1 x 100 µL

LeviSelect hCD8 Ab Cocktail (1 tube)
PN 6000050, 1 x 100 µL

1X LeviSelect Buffer (2 tubes)
PN 6000027, 2 x 1.8 mL

Additional Materials 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

Human CD8 T Cell Enrichment Data

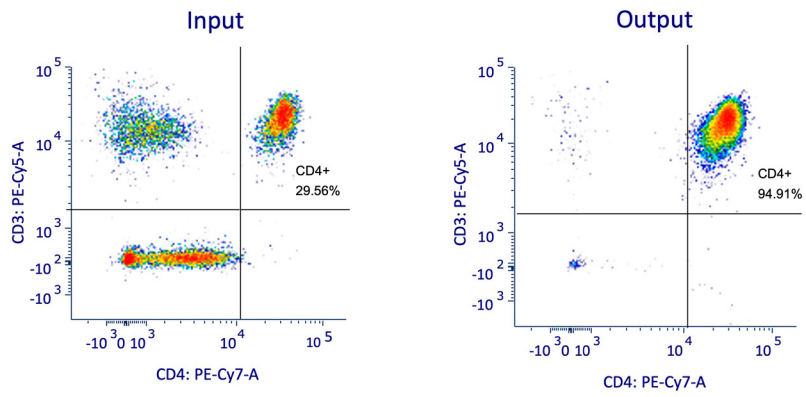

Figure 1. Human CD8+ T cell enrichment before and after running on the LeviCell 1.0 system. *2 x 10⁶ Human PBMCs were prepared for use with LeviSelect Human CD8 T Cell Enrichment Kit. After the LeviCell run, input and final output cells were stained using anti- CD3 PE-Cy5 and anti-CD8 PE-Cy5.5 to identify human CD8+ T cells.*

LeviSelect Human CD8 T Cell Enrichment Kit Protocol

A. Prepare Levitation Buffer

Reagent Volume	Volume for 1 sample (µL; includes 15% overage)	Volume for 4 samples (µL; includes 15% overage)
1X LeviSelect Buffer	131	526
Levitation Agent	49	194
Total	180	720

Table 1. Preparation of Levitation Buffer with final Levitation Agent concentration of 150mM

B. LeviCell Run with Human CD8 T Cell Enrichment

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 300RCF for 5 minutes. Remove and discard the supernatant. Resuspend cell pellet in 100 µL of 1X LeviSelect Buffer.

   NOTE: Antibody labeling of the sample prior to incubation with the antibody cocktail may disrupt binding of the cells to the nanospheres and inhibit depletion. It is recommended that all antibody labeling be performed after enrichment.
3. Pipet mix the tube containing the LeviSelect hCD8 Ab Cocktail.
4. Add 10 µL of hCD8 Ab Cocktail to the resuspended cells. Pipette mix with >80 µL 10 times. Total volume is now 110 µL.
5. Incubate cell suspension with the LeviSelect hCD8 Ab Cocktail for 15 minutes on ice.
6. Pipet mix the tube containing the LeviSelect hCD8 SAV Nanospheres.
7. Add 10 µL of hCD8 SAV Nanospheres to the resuspended cells with the antibody cocktail.
8. Incubate cell suspension with the LeviSelect hCD8 SAV Nanospheres for 15 minutes at room temperature.

   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.
9. Add 150 µL of prepared Levitation Buffer to the 2 mL tube. Total volume is now 270 µL.
10. Pipette mix with the same pipet tip 10 times. The magnetic beads should be uniformly dispersed throughout the solution
11. Set up the LeviCell cartridge on the instrument following the instructions on the Experiment Manager User Interface, selecting the “Standard” option.
12. 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 step.
13. Start the LeviCell run.

Reference LeviCell User Guide (#90-00204) for system instructions.