MojoSort™ Human CD8 Memory T Cells Isolation Kit Protocol

Kelsey Knight

Abstract

The kit is designed for the isolation of human memory CD8 T cells from PBMC. Target cells are depleted by incubating the sample with the biotin antibody cocktail followed by incubation with magnetic Streptavidin Nanobeads. The magnetically labeled fraction is retained by the use of a magnetic separator. The untouched cells are collected. These are the cells of interest; do not discard the liquid. Some of the downstream applications include functional assays, gene expression, phenotypic characterization, etc.

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Guidelines

MojoSort™ magnetic particles can be used with other commercially available magnetic separators, both free standing magnets and column-based systems. Because MojoSort™ protocols are optimized for the MojoSort™ separator; the protocols may need to be adjusted for other systems. Please contact BioLegend Technical Service (tech@biolegend.com) for more information and guidance. We do not recommend using MojoSort™ particles for BD's IMag™ or Life Technologies' DynaMag™.

Protocol

Step 1.

Prepare cells from your tissue of interest without lysing erythrocytes.

Step 2.

In the final wash of your sample preparation, resuspend the cells in MojoSort™ Buffer by adding up to 4mL in a 5mL (12 x 75 mm) polypropylene tube.

Note: Keep MojoSort™ Buffer on ice throughout the procedure.

Step 3.

Filter the cells with a 70µm cell strainer, centrifuge at 300xg for 5 minutes, and resuspend in an appropriate volume of MojoSort™ Buffer. Count and adjust the cell concentration to 1 x 10^8 cells/mL.

Step 4.

Aliquot 100µL of cell suspension (10^7 cells) into a new tube. Add 10µL of the Biotin-Antibody Cocktail, mix well and incubate on ice for 15 minutes. Scale up the volume accordingly if separating more cells. For example, add 100µL for 1 x 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7cells.

Optional: Keep unused cells, or take an aliquot before adding the cocktail to monitor purity and yield.

Step 5.

Resuspend the beads by vortexing, maximum speed, 5 touches. Add 10µL of Streptavidin Nanobeads. Mix well and incubate on ice for 15 minutes. Scale up the volume accordingly if separating more cells. For example, add 100µL for 1 x 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7cells.

Step 6.

Wash the cells by adding 3mL of MojoSort™ Buffer; centrifuge at 300xg for 5 minutes, discard supernatant.

Optional: Take an aliquot before placing the tube in the magnet to monitor purity and yield.

Step 7.

Resuspend the cells in 3mL of MojoSort™ Buffer.

Note: If you observe aggregates, filter the suspension. To maximize yield, you can disrupt the aggregates by pipetting the solution up and down.

Step 8.

Place the tube in the magnet for 5 minutes.

Step 9.

Pour out and collect the liquid. These are the cells of interest; **DO NOT DISCARD**.

Step 10.

If needed, add 3mL of MojoSort™ Buffer and repeat steps 8 and 9 with the magnetically labeled fraction up to two times, and then pool the unlabeled fractions.

Note: Repeating the magnetic separation increases the yield, without a strong impact on the purity. The yield will typically increase about 8-10% with a second separation, and about 2-5% with a third separation. The purity may decrease 1-2% with each separation.

Optional: Take a small aliquot before placing the tube in the magnet to monitor purity and yield.