MojoSort™ Human CD45 Nanobeads Protocol B

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Abstract

Note: If the percentage of CD45+ cells in your sample is less than 50%, please follow Protocol A. If it is higher than 50% then please follow protocol B.

The cells targeted by the Nanobeads are either selected or depleted by incubating your sample with the directly conjugated magnetic particles. The magnetically labeled fraction is retained by the use of a magnetic separator. After collection of the targeted cells, downstream applications include functional assays, gene expression, phenotypic characterization, etc.

Citation: Kelsey Miller MojoSort™ Human CD45 Nanobeads Protocol B. **protocols.io** dx.doi.org/10.17504/protocols.io.e22bgge
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Guidelines

This procedure is optimized for the isolation of 10^7 to 2 x 10^8 cells per tube. If working with fewer than 10^7 cells, keep volumes as indicated for 10^7 cells. For best results, optimize the conditions to your specific cell number and tissue. Prepare fresh MojoSort™ Buffer solution by diluting the 5X concentrate with sterile distilled water.

Reagents and instruments required:

-MojoSort™ Buffer (5X) (Cat. No. 480017)
-MojoSort™ Magnet (Cat. No. 480019) or compatible magnetic separation system
-Adjustable pipettes
-70 µm filters (one per sample)
-5 mL (12 x 75 mm) polystyrene tubes
-Reagents for sample preparation
-Reagents and instruments (Flow cytometer) to determine yield and purity

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 4 mL in a 5 mL (12 x 75 mm) polystyrene tube.

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

Step 3.

Filter the cells with a 70 µm cell strainer, centrifuge at 300 x g 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.

DURATION
00:05:00

Step 4.

Aliquot 100 µL of cell suspension (10^7 cells) into a new tube.

Step 5.

Resuspend the beads by vortexing, maximum speed, 5 touches. Add 10 µL of Nanobeads, mix well and incubate on ice for 15 minutes.

Scale up the volume accordingly if separating more cells. For example, add 100 µL for 1 x 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

DURATION
00:15:00

Step 6.

Add MojoSort™ Buffer up to 4 mL and centrifuge the cells at 300 x g for 5 minutes.

DURATION
00:05:00

Step 7.

Resuspend the cells in 3 mL of MojoSort™ Buffer.

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

Step 8.

Place the tube in the magnet for 5 minutes.

DURATION
00:05:00

Step 9.

Pour out the liquid containing the unlabeled fraction.

Step 10.

Remove the tube from the magnet and resuspend the first labeled fraction in appropriate amount of buffer.

Step 11.

Place the tube containing the unlabeled fraction back in the magnet for 5 minutes.

DURATION
00:05:00

Step 12.

Pour out the liquid containing the **unlabeled** fraction from the second magnetic incubation. These are the CD45- cells, ready to use as needed.

Step 13.

Remove the tube from the magnet and use the fraction obtained in step 10 to resuspend this second labeled fraction and pool them together. These are the CD45+ cells, ready to use as needed.

Optional: Take a small aliquot to monitor purity and yield. If desired, pool the unlabeled fractions and process simultaneously with the positive labeled cells when assessing purity and yield.