MojoSort™ Isolation Kits No Wash Protocol

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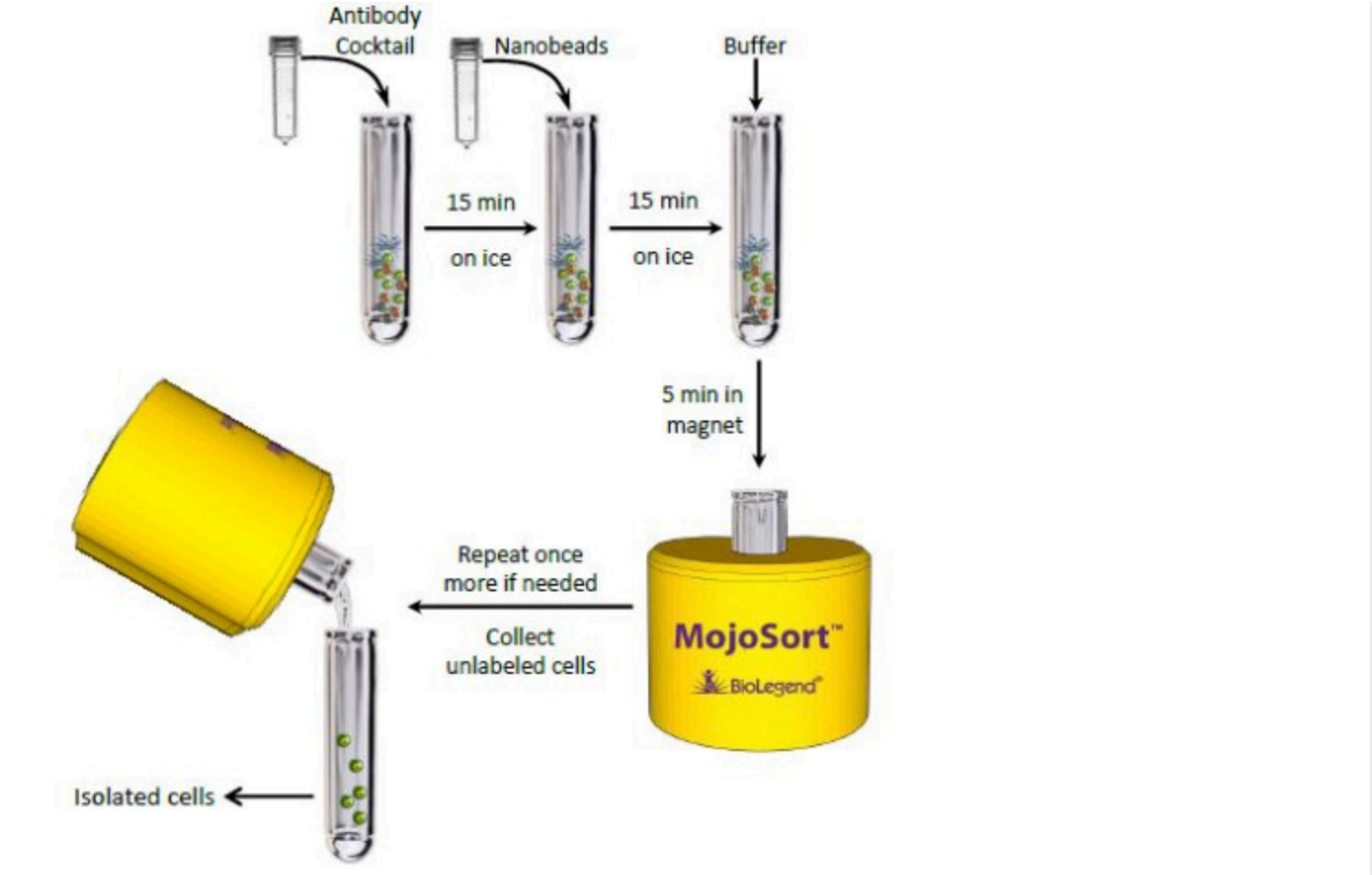
Abstract

Target cells are depleted by incubating your sample with the biotin antibody cocktail followed by incubation with magnetic Streptavidin Nanobeads (Cat. No. 480015/480016). The magnetically labeled fraction is retained by the use of a magnetic separator. The untouched cells are collected. These are your cells of interest; do not discard the liquid. Some of the downstream applications include functional assays, gene expression, phenotypic characterization, etc.

Guidelines

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 × 75 mm) polystyrene tubes
-Reagents for sample preparation
-Reagents and instruments (Flow cytometer) to determine yield and purity

Protocol:
This protocol has been optimized to remove washing steps after antibody cocktail and nanobeads incubations, resulting in a shorter and more convenient protocol. This procedure is optimized for the isolation of 10^7 to 2 × 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.



Troubleshooting

1 Prepare cells from your tissue of interest without lysing erythrocytes

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 × 75 mm) polystyrene tube.
Note: Keep MojoSort™ Buffer on ice throughout the procedure

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 × 10^8 cells/mL.
00:05:00

4 Aliquot 100 µL of cell suspension (10^7 cells) into a new tube. Add 10 µL 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 100 µL for 1 × 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.
Optional: Take an aliquot before adding the cocktail to monitor purity and yield.
00:15:00

5 Resuspend the beads by vortexing, maximum speed, 5 touches. Without washing, add 10 µL of Streptavidin 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 × 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells
00:15:00

6 Add 3 mL 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.

7 Place the tube in the magnet for 5 minutes
00:05:00

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

9 If needed, add 3 mL of MojoSort™ Buffer and repeat steps 7 and 8 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. Keep unused cells to be used as control or other applications if needed.