MojoSort™ Streptavidin Nanobeads Protocol

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Abstract

The cells targeted by the Streptavidin Nanobeads are either selected or depleted by incubating your sample with the magnetic particles after incubating with a biotin-conjugated antibody or antibody cocktail. 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.

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Guidelines

Protocol:

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

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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 108 cells/mL.

DURATION
00:05:00

Step 4.

Aliquot 100 µL of cell suspension (107 cells) into a new tube

Step 5.

Add appropriate amount of antibody or antibody cocktail to the cell suspension, mix well and incubate on ice for 15 minutes.

Note: The antibody volume to add should not exceed more than 20% of the 100 µL cell suspension volume. Thus, for 100 µL of cell suspension do not add more than 20 µL of antibody. If you need to add more than 20 µL of antibody, resuspend the cells in step 3 at a higher concentration. For example, to add 50 µL of antibody, resuspend the cells to a final concentration of 2 x 108 cells/mL. You can then aliquot 50 µL of cells and add 50 µL of antibody, mix well and incubate on ice for 15 minutes. Always keep the total volume around 100 µL.

DURATION
00:15:00

Step 6.

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

DURATION
00:05:00

Step 7.

Resuspend the cells in 100 µL of of MojoSort™ Buffer

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Step 8.

Resuspend the beads by vortexing, maximum speed, 5 touches. Add appropriate amount of pre-titrated Streptavidin Nanobeads, mix well and incubate on ice for 15 minutes.

Note: The Streptavidin Nanobeads volume to add should not exceed more than 20% of the 100 µL cell suspension volume. Thus, for 100 µL of cell suspension do not add more than 20 µL of Nanobeads. If you need to add more than 20 µL of Nanobeads, resuspend the cells in step 6 at a higher concentration. For example, to add 50 µL of Nanobeads, resuspend the cells in 50 µL of MojoSort™ Buffer. Always keep the total volume around 100 µL.

DURATION
00:15:00

Step 9.

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

DURATION
00:05:00

Step 10.

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 11.

Place the tube in the magnet for 5 minutes

DURATION
00:05:00

Step 12.

Negative Selection: If you are interested in the untouched cells, collect the liquid in a new tube. These are your cells of interest; do not discard.

If needed, repeat the magnetic separation on this cell fraction to increase the yield.

Positive Selection: If you are interested in the cells that are bound to the Nanobeads, pour off the liquid while the tube is in the magnet (negative fraction). Then, remove the tube from the magnet and collect your cells. Repeat steps 9 – 11 on the labeled fraction 2 more times, for a total of 3 magnetic separations to increase yield, if needed.

Optional: Take a small aliquot to monitor purity and yield. Pool the unlabeled fractions and process

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simultaneously with the positive labeled cells when assessing purity and yield.

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