MojoSort™ Mouse CX3CR1 Selection Kit Protocol

Kelsey Miller

Abstract

This procedure is optimized for the isolation of 10^6 cells per test. If working with fewer than 10^6 cells, keep volumes as indicated for 10^6 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.

Product description and procedure summary:
Target cells are positively selected or depleted by incubating the sample with the biotin anti-mouse CX3CR1
antibody followed by incubation with magnetic Streptavidin Nanobeads. The magnetically labeled fraction is
retained by the use of a magnetic separator. These are the CX3CR1+ cells, do not discard them if those are the
cells of interest. Some of the downstream applications include functional assays, gene expression, phenotypic
characterization, etc.

Citation: Kelsey Miller MojoSort™ Mouse CX3CR1 Selection Kit Protocol. **protocols.io**
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Guidelines

Reagents and instruments required:
MojoSort™ Buffer (5X) (Cat. No. 480017)
MojoSort™ Magnet (Cat. No. 480019/480020) or compatible magnetic separation system
Adjustable pipettes
70 µm filters (one per sample)
5 mL (12 x 75 mm) or 14 mL (17 x 100 mm) polystyrene tubes
Reagents for sample preparation
Reagents and instruments (Flow cytometer) to determine yield and purity

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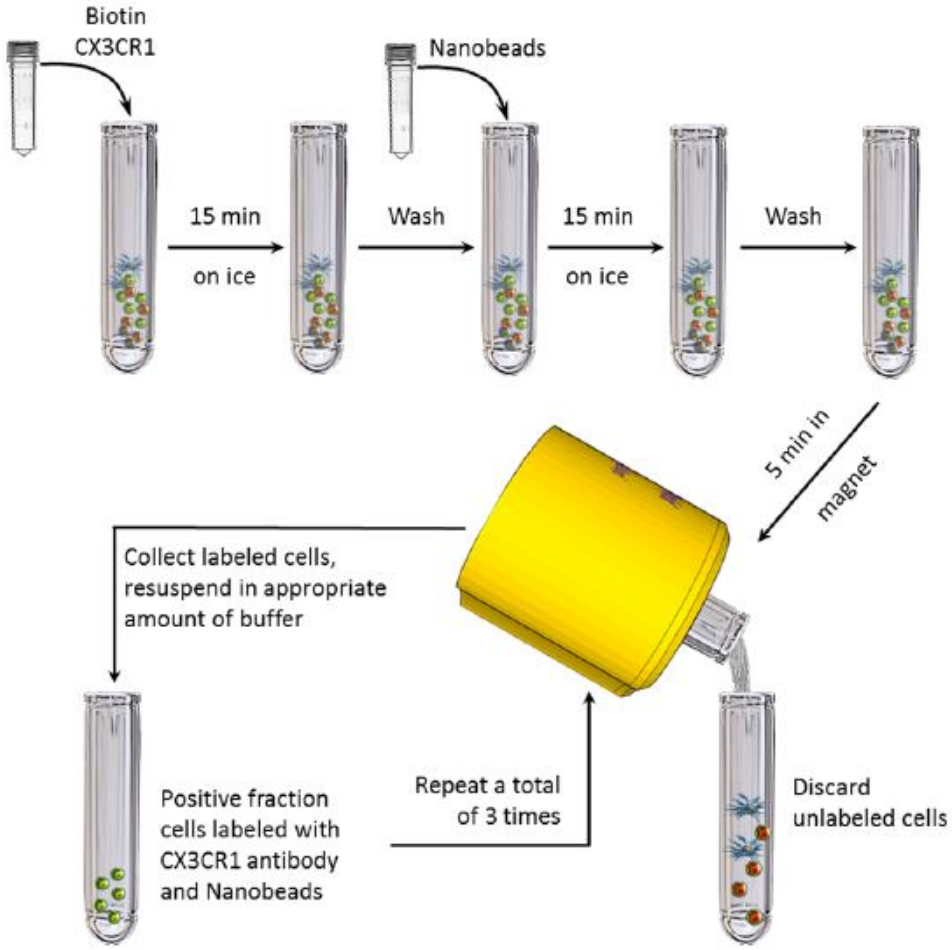
Chart Protocol:



Application notes: To use this product in magnetic separation columns, a titration of the cocktail/beads should be performed. Optimal concentration for magnetic separation columns is lot-specific. Please contact BioLegend Technical Service (tech@biolegend.com) for further assistance on how to use MojoSort™ Nanobeads in magnetic separation columns.

Protocol

Step 1.

Prepare cells from your tissue of interest. Enzymatic digestion, followed by myelin removal, is strongly recommended.

Step 2.

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

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

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Step 3.

Filter the cells with a 40 µm cell strainer, centrifuge at 300 x g for 5 minutes, and resuspend in anappropriate volume of MojoSort™ Buffer.

Count and adjust the cell concentration to 1 x 10^7cells/mL.

DURATION
00:05:00

Step 4.

Aliquot 100 µL of cell suspension (10^6 cells) into a new tube. Add 10 µL of the Biotin-CX3CR1 antibody, 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 10^7 cells. When working with less than 10^6 cells, use indicated volumesfor 10^6 cells.

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

DURATION
00:15:00

Step 5.

Wash the cells by adding MojoSort™ Buffer up to 4 mL; centrifuge the cells at 300 x g for 5 minutes.

DURATION
00:05:00

Step 6.

Discard supernatant and resuspend in 100 µL of MojoSort™ Buffer.

Step 7.

Resuspend the beads by vortexing, maximum speed, 5 touches. 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 x 10^7 cells. When working with less than 10^6 cells, use indicated volumes for 10^6 cells.

DURATION
00:15:00

Step 8.

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

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

DURATION
00:05:00

Step 9.

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Resuspend the cells in 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.

Step 10.

Place the tube in the magnet for 5 minutes.

DURATION
00:05:00

Step 11.

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

Step 12.

If needed, add 3 mL of MojoSort™ Buffer and repeat steps 10 and 11 with the magnetically labeled fraction up to two times, and then pool the unlabeled fractions.

Notes: 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 beforeplacing the tube in the magnet to monitor purity and yield. Scale up volumes accordingly if using a 14mL tube.

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