MagIso™ CD8 T Cell Isolation Kit, Mouse

Cat.No: WHK-B004

DESCRIPTION

Description	Non $CD8^+$ T cells are depleted by incubating your sample with the biotin antibody cocktail followed by incubation with magnetic Streptavidin Nanoparticles. The magnetically labeled fraction is retained by the use of a magnetic separator. The untouched $CD8^+$ T cells are collected by decanting the liquid in a clean tube. These are your cells of interest; do not discard the liquid. Some of the downstream applications include functional assays, gene expression, phenotypic characterization, etc.

APPLICATION

Application Notes	This kit is designed for the isolation of untouched $CD8^+$ T cells from lymphoid tissues.

KIT COMPONENTS

Kit Components	Quantity	Storage
Streptavidin magnetic nanoparticles	100 μL-10 tests/1 mL-100 tests	2-8 °C
Biotin-Antibody Cocktail: Biotin anti- CD4, CD11b, CD11c, CD19, CD24, CD45/B220, CD49b, CD105, I-A/I-E (MHC II), TER-119/Erythroid, TCR-γδ	100 μL-10 tests/1 mL-100 tests	2-8 °C

PRODUCT INFORMATION

Species Reactivity	Mouse

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Isolation Method	Negative Selection
Sample Type	Lymphoid Tissues
Target Cell	$CD8^+$ T cells

MATERIALS

Materials Required But Not Supplied	• Cell Isolation Buffer(5X): 5X Phosphate buffered saline (PBS), pH 7.2; 2.5 % (w/v) Bovine Serum Albumin (BSA); 10 mM Ethylene Diamine Tetra-acetic acid (EDTA). • 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

Experimental Procedure

Experimental Procedure

Protocol 1

This procedure is optimized for the isolation of 10^7 to 2 x 10^8 cells per tube from lymphoid organs. 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. If you are using an automatic separator instrument that is not compatible with the washing steps, you can omit steps 5 and 8 without a big impact on purity and yield. You can also omit these steps if you need a shorter protocol. The yield and purity increase by 2-5% with the washes.

Prepare fresh Cell Isolation Buffer solution by diluting the Cell Isolation Buffer(5X) with sterile distilled water. The 5X concentrate is filtered through a 0.2 µm membrane. Use the buffer under aseptic conditions as needed.

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1. Prepare cells from your tissue of interest without lysing erythrocytes.
2. In the final wash of your sample preparation, resuspend the cells in Cell Isolation Buffer by adding up to 4 mL in a 5 mL (12 x 75 mm) polystyrene tube.
Note: Keep Cell Isolation 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 Cell Isolation Buffer. Count and adjust the cell concentration to 1 x 10^8 cells/mL.
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 x 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.
Optional: Keep unused cells, or take an aliquot before adding the cocktail to monitor purity and yield.
5. Wash the cells by adding Cell Isolation Buffer up to 4 mL; centrifuge the cells at 300 x g for 5 minutes.
6. Discard supernatant and resuspend in 100 µL of Cell Isolation Buffer.
7. Resuspend the particles by vortexing, maximum speed, 5 touches. Add 10 µL of Streptavidin Nanoparticles. 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.
8. Wash the cells by adding 3 mL of Cell Isolation 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.
9. Resuspend the cells in 3 mL of Cell Isolation Buffer.
Note: If you observe aggregates, filter the suspension. To maximize yield, you can disrupt the aggregates by pipetting the solution up and down.
10. Place the tube in the magnet for 5 minutes.
11. Pour out and collect the liquid. These are your cells of interest; DO NOT DISCARD.
12. If needed, add 3 mL of Cell Isolation Buffer and repeat steps 10 and 11 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

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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.

Protocol 2. No Wash Protocol

This protocol has been optimized to remove washing steps after antibody cocktail and Nanoparticles incubations, resulting in a shorter and more convenient protocol. This procedure is optimized for the isolation of 10^7 to 2 x 10^8 cells per tube from lymphoid organs. 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.

1. Prepare cells from your tissue of interest without lysing erythrocytes.
2. In the final wash of your sample preparation, resuspend the cells in Cell Isolation Buffer by adding up to 4 mL in a 5 mL (12 x 75 mm) polystyrene tube.
Note: Keep Cell Isolation 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 Cell Isolation Buffer. Count and adjust the cell concentration to 1 x 10^8 cells/mL.
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 x 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.
Optional: Keep unused cells, or take an aliquot before adding the cocktail to monitor purity and yield.
5. Resuspend the particles by vortexing, maximum speed, 5 touches. Without washing, add 10 µL of Streptavidin Nanoparticles. 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.
6. Resuspend the cells in 3 mL of Cell Isolation Buffer.
Note: If you observe aggregates, filter the suspension. To maximize yield, you can disrupt the aggregates by pipetting the solution up and down.

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7.Place the tube in the magnet for 5 minutes.
8.Pour out and collect the liquid. These are your cells of interest; DO NOT DISCARD.
9.If needed, add 3 mL of Cell Isolation 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 beforeplacing the tube in the magnet to monitor purity and yield.

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