MagIso™ Pan Monocyte Cell Isolation Kit, Human Protocol

WHK-B021

Reagents Required

- Streptavidin magnetic nanoparticles
- Biotin-antibody mixture
- Human Fc receptor blocking solution
- Buffer Solution: 5X Phosphate buffered saline (PBS), pH 7.2, with 2.5 % w/v BSA, 10 mM EDTA

Note:

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 Buffer Solution by diluting the 5X concentrate with sterile distilled water. Scale up volumes if using 14 mL tubes and Magnet, and place the tube in the magnet for 10 minutes.

Sample Preparation:

It is strongly recommended that platelets be removed prior to the isolation of monocytes using a suitable method. See recommended platelet removal protocol below.

Platelet Removal Protocol

1. Dilute blood with 2-4 times (volume/volume) 1X PBS.

2. Carefully layer diluted blood over 12.5mL of isolation medium in a 50mL tube.

3. Centrifuge at 400xg for 25 minutes at room temperature in a swinging-bucket rotor without the brake.

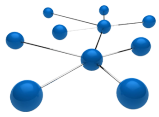
4. Aspirate the upper layer of the gradient (serum), leaving the interphase containing the mononuclear cells undisturbed.

5. Carefully transfer the mononuclear cells to a new 50mL tube.

6. Fill the tube with 1X PBS, mix, and centrifuge at 200xg for 8 minutes at room temperature. Carefully remove supernatant as much as possible.

7. Repeat step 6.

8. Proceed to separation protocol.



Separation Protocol

1. In the final wash of your sample preparation, resuspend the cells in Buffer Solution by adding up to 4mL in a 5 mL polypropylene tube.

Note: Keep Buffer Solution on ice throughout the procedure.

2. Filter the cells with a 70 µm cell strainer, centrifuge at 300xg for 5 minutes, and resuspend in an appropriate volume of Buffer Solution. Count and adjust the cell concentration to 1 x 10^8 cells/mL.

3. Aliquot 100 µL of cell suspension (10^7 cells) into a new tube. Add 5 µL of Human Fc Receptor Blocking Solution, mix well and incubate at room temperature for 10 minutes. Scale up the volume accordingly if separating more cells. For example, if the volume of Blocking Solution for 1x10^7 cells is 5µL, add 50µL for 1 x 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

4. Add 10 µL of the Biotin-antibody mixture. Mix well and incubate on ice for 15 minutes. Scale up the volume accordingly if separating more cells. For example, add 100 µL of Biotin-antibody mixture for separating 1 x 10^8 cells in 1 ml of Buffer Solution. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

Optional: Take an aliquot before adding the Biotin-antibody mixture to monitor purity and yield.

5. Resuspend the beads by vortexing, maximum speed, 5 touches. Add 10µL of Streptavidin magnetic particles. Mix well and incubate on ice for 15 minutes. Scale up the volume accordingly if separating more cells. For example, add 100 µL of Nanobeads for separating 1 x 10^8 cells in 1 ml of Buffer Solution. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

6. Wash the cells by adding Buffer Solution up to 4mL. Centrifuge the cells at 300xg for 5 minutes.

7. Discard supernatant.

8. Add 2.5mL of Buffer Solution.

Note: If you observe aggregates, filter the suspension. To maximize yield, you can disrupt the aggregates by pipetting the solution up and down.

9. Place the tube in the magnet for 5 minutes.

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.

10. Pour out and collect the liquid. These are your cells of interest; DO NOT DISCARD. Resuspend the labeled cells in 2.5mL Buffer Solution.

11. Repeat steps 8-10 on the labeled fraction once more for a total of 2 separations. Pool the unlabeled fractions. The labeled cells may be useful as staining controls, to monitor purity/yield, or other purposes.

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. The purity may decrease 1-2% with each separation.