Protocol: Absolute Mag™ Human Anit-CD45 Magnetic Nanoparticles, 130 nm

Material Required

- Buffer (5X): 5X Phosphate buffered saline (PBS), pH 7.2, with 2.5 % (w/v) Bovine Serum Albumin (BSA) and 10 mM Ethylene Diamine Tetra-acetic acid (EDTA)
- Magnet
- Adjustable pipettes
- 70 µm filters (one per sample)
- 5 mL (12 x 75mm) or 14 mL (17 x 100 mm) polypropylene tubes polypropylene tubes
- Reagents for sample preparation
- Reagents and instruments (Flow cytometer) to determine yield and purity

Note: If the percentage of CD45+ cells in your sample is less than 50%, please follow ***Protocol 1***. If it is higher than 50% then please follow ***Protocol 2***.

Protocol 1

The cells targeted by the Nanobeads are either selected or depleted by incubating your sample with the directly conjugated magnetic particles. 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.

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.

1. Prepare cells from your tissue of interest or blood without lysing erythrocytes. Kits for human samples have been optimized for PBMCs, please prepare the cells using a suitable method.

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

Note: Keep **Buffer** on ice throughout the procedure.

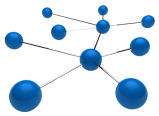
3. Filter the cells with a 70 µm cell strainer, centrifuge at 300xg for 5 minutes, and resuspend in an appropriate volume of **Buffer**. Count and adjust the cell concentration to 1 x 10^8 cells/mL.

4. Aliquot 100 µL of cell suspension (10^7 cells) into a new tube.

5. Resuspend the beads by vortexing, maximum speed, 5 touches. Add 10 µL of Antibody Nanobeads. 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**. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

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

7. Discard the supernatant.



8. Add 2.5 mL of **Buffer**.

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 the unlabeled fraction. If these are your cells of interest, DO NOT DISCARD. Resuspend the labeled cells in 2.5 mL **Buffer**.

11. Repeat steps 8-10 on the labeled fraction twice more for a total of 3 separations. Pool the unlabeled fractions and keep the labeled cells. The fraction that is not of interest may be useful as staining controls, to monitor purity/yield, or other purposes.

Optional: Take a small aliquot to monitor purity and yield.

Protocol 2

The cells targeted by the Nanobeads are either selected or depleted by incubating your sample with the directly conjugated magnetic particles. 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.

Note:

This procedure is optimized for the isolation of 10^7 to 2 x 10^8cells 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 14mL tubes and Magnet, and place the tube in the magnet for 10 minutes.

1. Prepare cells from your tissue of interest or blood without lysing erythrocytes. Kits for human samples have been optimized for PBMCs, please prepare the cells using a suitable method.

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

Note: Keep **Buffer** on ice throughout the procedure.

3. Filter the cells with a 70 µm cell strainer, centrifuge at 300xg for 5 minutes, and resuspend in an appropriate volume of **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.

5. Resuspend the beads by vortexing, maximum speed, 5 touches. Add 10 µL of 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 10^8 cells. When working with less than 10^7 cells, use indicated volumes for 10^7 cells.

6. Add **Buffer** up to 4 mL and centrifuge the cells at 300xg for 5 minutes.

7. Resuspend the cells in 3 mL of **Buffer**.

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

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

9. Pour out the liquid containing the unlabeled fraction.

10. Remove the tube from the magnet and resuspend the first labeled fraction in appropriate amount of buffer.

11. Place the tube containing the unlabeled fraction back in the magnet for 5 minutes.

12. Pour out the liquid containing the unlabeled fraction from the second magnetic incubation. These are the CD45- cells, ready to use as needed.

13. Remove the tube from the magnet and use the fraction obtained in step 10 to resuspend this second labeled fraction and pool them together. These are the CD45+ cells, ready to use as needed.

Optional: Take a small aliquot to monitor purity and yield. If desired, pool the unlabeled fractions and process simultaneously with the positive labeled cells when assessing purity and yield.

The protocol may need to be adjusted, depending on the experiment.