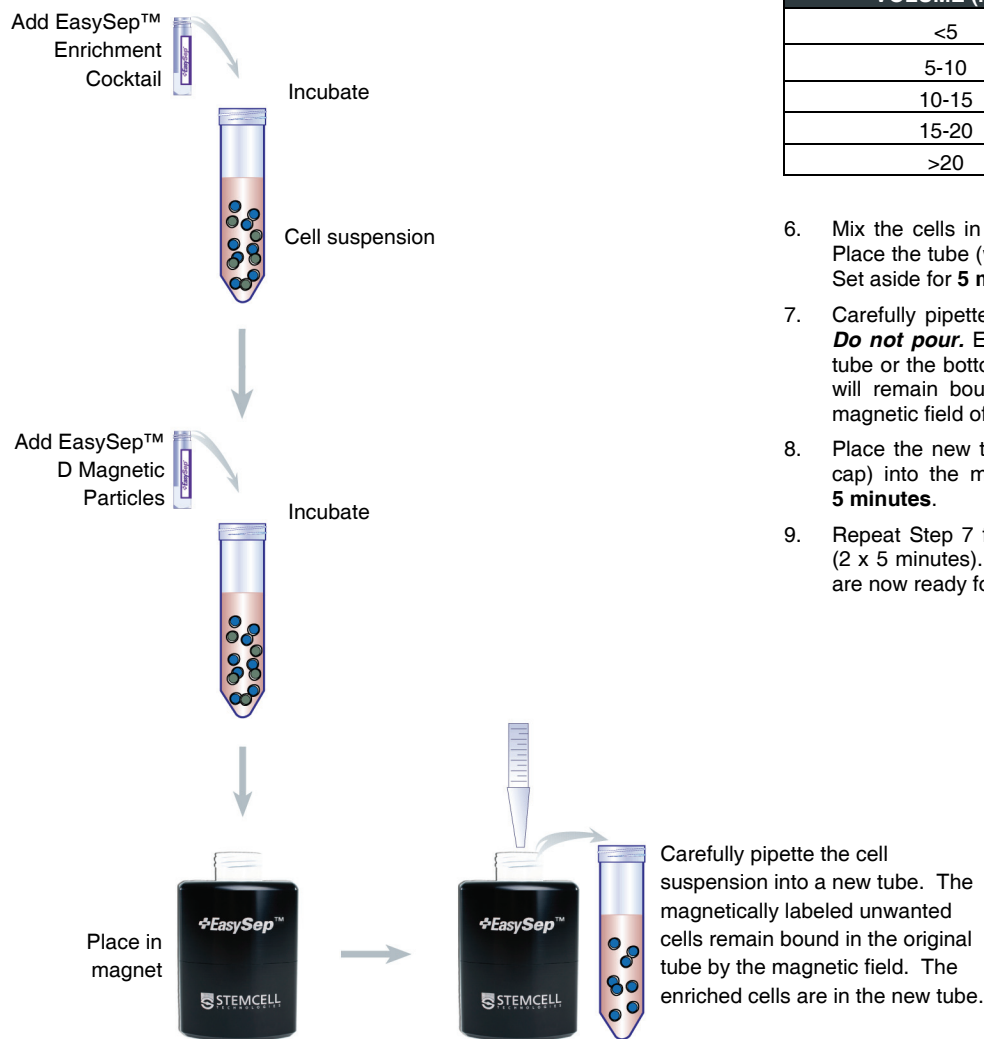


THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH THE "EASY 50" EASYSEP™ MAGNET (CATALOG #18002). FOR USE WITH OTHER EASYSEP™ MAGNETS, PLEASE REFER TO THE PRODUCT INFORMATION SHEET PACKAGED WITH THE KIT, OR VISIT WWW.STEMCELL.COM.

"EASY 50" EASYSEP™ PROTOCOL DIAGRAM



MANUAL EASYSEP™ PROTOCOL USING THE "EASY 50" EASYSEP™ MAGNET (CATALOG #18002)

FOR USE WITH:

- HUMAN PBMCs

This procedure is used for processing up to 35 mL of sample.

1. Prepare cell suspension at a concentration of 5×10^7 cells/mL in recommended medium (see Notes and Tips, reverse side). Cells must be placed in a 50 mL conical tube to properly fit into the "Easy 50" EasySep™ Magnet.
Falcon™ 50 mL conical tubes (BD Biosciences, Catalog #352070) are recommended.
2. Add the EasySep™ Human Pan-DC Pre-Enrichment Cocktail at **50 μ L/mL cells** (e.g. for 2 mL of cells, add 100 μ L of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **30 minutes**.
3. Vortex the EasySep™ D Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
4. Add the EasySep™ D Magnetic Particles at **250 μ L/mL cells** (e.g. for 2 mL of cells, add 500 μ L of particles). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.
5. Bring the cell suspension up to the **total volume** specified in Table 1, using recommended medium.

TABLE 1: RESUSPENSION VOLUMES

ORIGINAL SAMPLE VOLUME (mL)	TOTAL VOLUME (mL)
<5	10
5-10	20
10-15	30
15-20	40
>20	50

6. Mix the cells in the tube by gently pipetting up and down 2 - 3 times. Place the tube (without cap) into the magnet and push all the way down. Set aside for **5 minutes**.
7. Carefully pipette the enriched cell suspension into a new 50 mL tube. **Do not pour.** Ensure that the pipettor does not touch the sides of the tube or the bottom of the tube. The magnetically labeled unwanted cells will remain bound along the inside of the original tube, held by the magnetic field of the "Easy 50" EasySep™ Magnet.
8. Place the new tube containing the negatively selected fraction (without cap) into the magnet for a second round of separation. Incubate for **5 minutes**.
9. Repeat Step 7 for a total of two-five minute incubations in the magnet (2 x 5 minutes). The negatively selected, enriched cells in the new tube are now ready for use.

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NOTES AND TIPS

REQUIRED EQUIPMENT. “Easy50” EasySep™ Magnet (Catalog #18002).

RECOMMENDED MEDIUM. The recommended medium is RoboSep™ Buffer (Catalog #20104), or Phosphate Buffered Saline (PBS) + 2% FBS (Catalog #07905) with 1 mM EDTA. Medium should be Ca⁺⁺ and Mg⁺⁺ free.

SPECIFIED TUBE. The “Easy 50” EasySep™ Magnet is designed to hold a 50 mL conical tube (BD Biosciences, Catalog # 352070).

PREPARING THE CELL SUSPENSION**FROM WHOLE PERIPHERAL BLOOD**

Prepare a mononuclear cell suspension from whole peripheral blood (PBMC) by density gradient centrifugation. **For previously frozen mononuclear cells, we recommend incubating the cells with DNase I (Catalog #07900)** at a concentration of 100 µg/mL for at least 15 minutes at room temperature (15 - 25°C) prior to labeling and separation. Filter clumpy suspensions through a 30 µm mesh nylon strainer for optimal results.

BETWEEN 1 - 35 mL OF PBMC (UP TO 1.75 X 10⁹ CELLS) CAN BE USED IN THE “EASY 50” EASYSEP™ MAGNET AT ONE TIME.