

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

FOR USE WITH:

- HUMAN PBMCs
- AMMONIUM CHLORIDE-LYSED PERIPHERAL BLOOD APHERESIS

This procedure is used for processing up to 30 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 Naïve B Cell Enrichment Cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **10 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 µL/mL cells** (e.g. for 2 mL of cells, add 500 µL of particles). Mix well and incubate at room temperature (15 - 25°C) for **5 minutes**.
5. Bring the cell suspension up to the **total volume** specified in Table 1, using recommended medium.

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

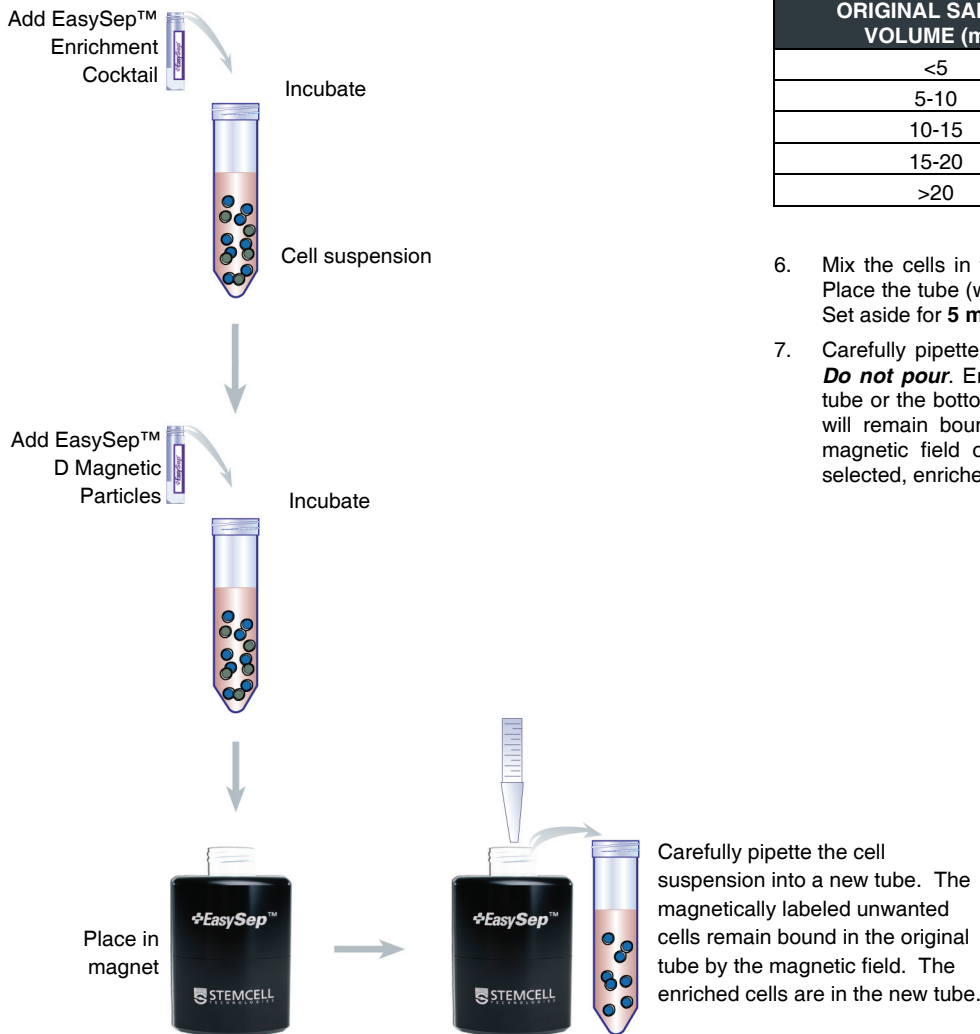


TABLE 1: RESUSPENSION VOLUMES

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. The negatively selected, enriched cells in the new tube are now ready for use.

WWW.STEMCELL.COM

FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC USE



IN NORTH AMERICA TOLL-FREE T. 1 800 667 0322 TOLL-FREE F. 1 800 567 2899 T. +1 604 877 0713 F. +1 604 877 0704 E. INFO@STEMCELL.COM
 IN EUROPE TOLL-FREE T. 00 800 7836 2355 TOLL-FREE F. 00 800 7836 2300 E. INFO.EU@STEMCELL.COM
 IN AUSTRALIA TOLL-FREE T. 1 800 060 350 F. +61 (03) 9338 4320 E. INFO.AUS@STEMCELL.COM
 IN SINGAPORE T. +(65) 6776 7754 F. +(65) 6776 7114 E. INFO.SG@STEMCELL.COM

NOTES AND TIPS

REQUIRED EQUIPMENT. “Easy 50” 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 - 30 mL OF PBMC (UP TO 1.5 X 10⁹ CELLS) CAN BE USED IN THE “EASY 50” EASYSEP™ MAGNET AT ONE TIME.

FROM PERIPHERAL BLOOD APHERESIS (LEUKOPAK)

If working with large volumes (>150 mL), concentrate Leukopak cells first by centrifuging at 500 x *g* for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original Leukopak volume with recommended medium (e.g. for 300 mL of cells, resuspend in 30 mL of recommended medium). For small volumes (150 mL or less), add the Ammonium Chloride Solution (Catalog #07800/07850) directly to the cell suspension.

1. Add an equal volume of Ammonium Chloride Solution to the Leukopak suspension. (e.g. for 5 mL of Leukopak suspension, add 5 mL Ammonium Chloride Solution).
2. Incubate 15 minutes on ice.
3. Centrifuge at 500 x *g* for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
4. Wash the cells by topping up the tube with the recommended medium. Centrifuge at 150 x *g* for 10 minutes at room temperature (15 - 25°C) with the brake off. Carefully remove the supernatant.
5. Repeat the wash step one or more times until most of the platelets have been removed (indicated by a clear supernatant).
6. Resuspend cells at 5 x 10⁷ cells/mL in recommended medium.

BETWEEN 1 - 30 mL OF LYSED LEUKAPHERESIS (UP TO 1.5 X 10⁹ CELLS) CAN BE USED IN THE “EASY 50” EASYSEP™ MAGNET AT ONE TIME.