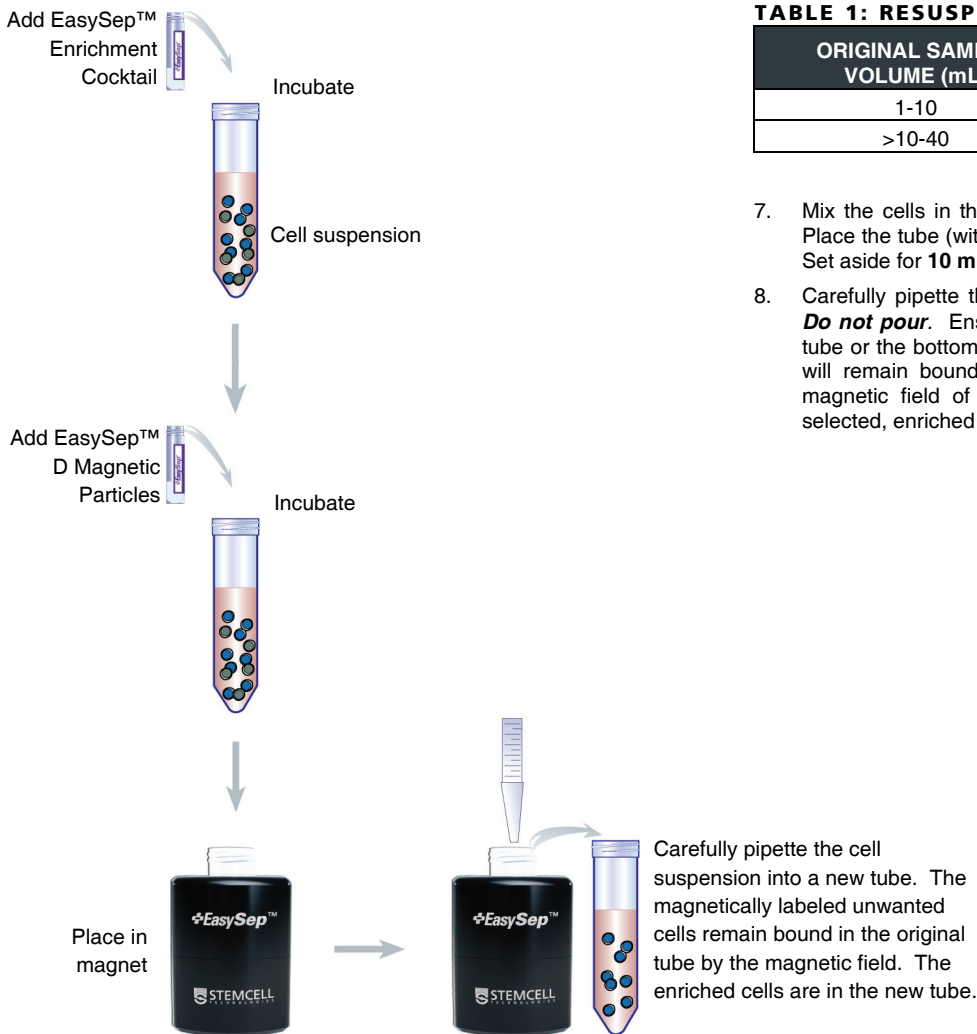


**THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH THE "EASY 50" EASYSEP™ MAGNET (CATALOG #18002). FOR USE WITH OTHER EASYSEP™ MAGNETS, PLEASE REFER TO THE PRODUCT INFORMATION SHEET PACKAGED WITH THE KIT, OR VISIT WWW.STEMCELL.COM.**

**"EASY 50" EASYSEP™ PROTOCOL DIAGRAM**



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

- FOR USE WITH:**
- **MOUSE SPLEEN**

This procedure is used for processing up to 40 mL of sample.

1. Prepare cell suspension at a concentration of  $1 \times 10^8$  cells/mL in recommended medium (see Notes and Tips, reverse side). Cells must be placed in a 50 mL conical tube to fit properly into the "Easy 50" EasySep™ Magnet.  
*Falcon™ 50 mL conical tubes (BD Biosciences, Catalog #352070) are recommended.*
2. Add the EasySep™ Mouse 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 in the refrigerator (2 - 4°C) for **15 minutes**.
3. Add EasySep™ Biotin Selection Cocktail at **100 µL/mL cells** (e.g. for 2 mL of cells, add 200 µL of selection cocktail). Mix well and incubate in the refrigerator (2 - 4°C) for **15 minutes**.
4. Vortex the EasySep™ D Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
5. Add the EasySep™ D Magnetic Particles at **100 µL/mL cells** (e.g. for 2 mL of cells, add 200 µL of particles). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.
6. 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)
1-10	To 25 mL
>10-40	To 50 mL

7. 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 **10 minutes**.
8. 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.

**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<sup>++</sup> and Mg<sup>++</sup> 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**

**PREPARING A SINGLE CELL SUSPENSION FROM MOUSE SPLEEN**

Disrupt the spleen in 5 mL Phosphate Buffered Saline (PBS) or Hank’s Balanced Salt Solution (HBSS) plus Fetal Bovine Serum (FBS) + 2% FBS. Centrifuge at 300 x g for 10 minutes and resuspend cells at 1 x 10<sup>8</sup> nucleated cells/mL in the recommended medium. Ammonium chloride treatment is not recommended when preparing the cells for separation.

**BETWEEN 1 - 40 mL OF MOUSE SPLEEN SAMPLE (UP TO 4 X 10<sup>9</sup> CELLS) CAN BE USED IN “THE EASY 50” EASYSEP™ MAGNET AT ONE TIME.**