

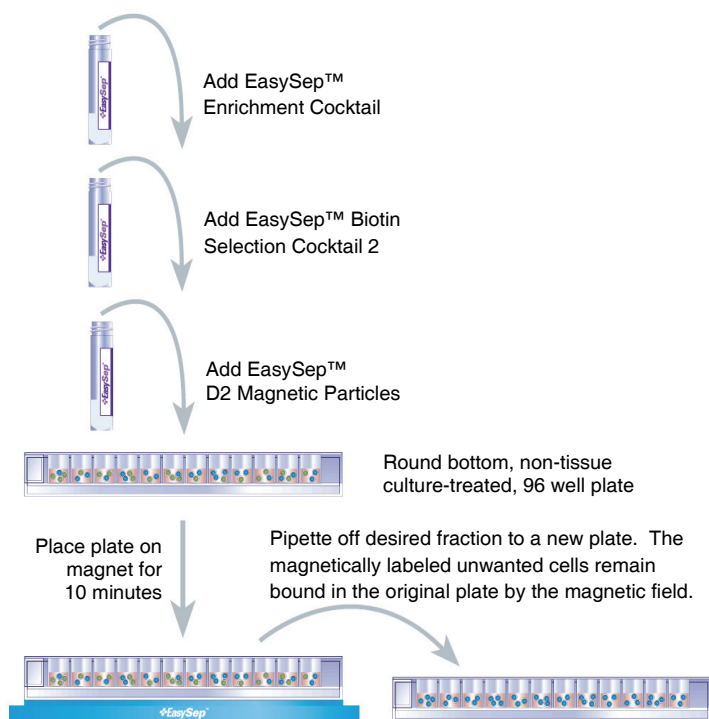
MANUAL EASYSEP™ PROTOCOL USING THE "EASYPLATE" EASYSEP™ MAGNET (CATALOG #18102)

This procedure is used for processing **50 µL - 200 µL** of sample per well (up to 2×10^7 cells per well or 1.92×10^9 cells per 96 well plate). For volumes less than 50 µL, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH THE "EASYPLATE" MAGNET (CATALOG #18102). FOR USE WITH OTHER EASYSEP™ MAGNETS, PLEASE REFER TO THE PRODUCT INFORMATION SHEET PACKAGED WITH THE KIT, OR VISIT WWW.STEMCELL.COM.

1. Prepare cell suspension at a concentration of 1×10^8 cells/mL in recommended medium (see Notes and Tips, reverse side). Add the Normal Rat Serum (provided) at **50 µL/mL cells** (e.g. for 200 µL of cell suspension, add 10 µL of rat serum). Cells must be placed in a round bottom, non-tissue culture-treated 96 well plate that will properly fit on the "EasyPlate" Magnet (see Notes and Tips, reverse side).
2. Add the EasySep™ Mouse CD4⁺ T Cell Enrichment Cocktail at **50 µL/mL cells** (e.g. for 200 µL of cells, add 10 µL of cocktail). Mix well and incubate in refrigerator (2 - 8°C) for **15 minutes**.
3. Add the EasySep™ Biotin Selection Cocktail 2 at **100 µL/mL cells** (e.g. for 200 µL of cells, add 20 µL of selection cocktail). Mix well and incubate in refrigerator (2 - 8°C) for **15 minutes**.
4. Vortex the EasySep™ D2 Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
5. Add the EasySep™ D2 Magnetic Particles at **100 µL/mL cells** (e.g. for 200 µL of cells, add 20 µL of particles). Mix well and incubate in refrigerator (2 - 8°C) for **5 minutes**.
6. Bring the cell suspension up to a **total volume of 250 µL per well** using recommended medium. Mix the cells in the well by gently pipetting up and down 2 - 3 times.
7. Place the 96 well plate onto the "EasyPlate" EasySep™ Magnet, ensuring that the plate sits securely on the magnet. Incubate for **10 minutes**.
8. Carefully pipette the enriched cell suspension from each well into a new 96 well plate. **Do not pour**. The magnetically labeled unwanted cells will remain bound to the bottom of the original well, held by the magnetic field of the "EasyPlate" EasySep™ Magnet. The negatively selected, enriched cells in the new 96 well plate are now ready for use.

"EASYPLATE" EASYSEP™ PROTOCOL DIAGRAM



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NOTES AND TIPS

REQUIRED EQUIPMENT. “EasyPlate” EasySep™ Magnet (Catalog #18102).

RECOMMENDED MEDIUM. The recommended medium is RoboSep™ Buffer (Catalog #20104), or Phosphate Buffered Saline (PBS) + 2% FBS (Catalog #07905) with 1 mM EDTA. Hanks’ Balanced Salt Solution (Hanks’ BSS) (Catalog #37250) can be used in place of PBS (Catalog #37350). Medium should be Ca⁺⁺ and Mg⁺⁺ free.

RECOMMENDED 96 WELL PLATE. The “EasyPlate” EasySep™ Magnet is designed to hold a 96 well plate (such as Costar, Catalog #3788 or BD Biosciences, Catalog #351177). Round bottom, non-tissue culture-treated plates work best.

If using a different type of non-tissue culture treated 96 well plate, ensure that it properly fits on the “EasyPlate” EasySep™ Magnet before use. Some 96 well plates may not sit flat on the magnet, which could affect the success of the separation.

PREPARING THE CELL SUSPENSION

PREPARING A SINGLE CELL SUSPENSION. Disrupt spleen in 5 mL Phosphate Buffered Saline (PBS) or Hank’s Balanced Salt Solution plus 2% Fetal Bovine Serum (FBS). Centrifuge at 300 x *g* for 10 minutes and resuspend at 1 x 10⁸ nucleated cells/mL in recommended medium. Ammonium chloride treatment is not recommended when preparing the cells for separation.