

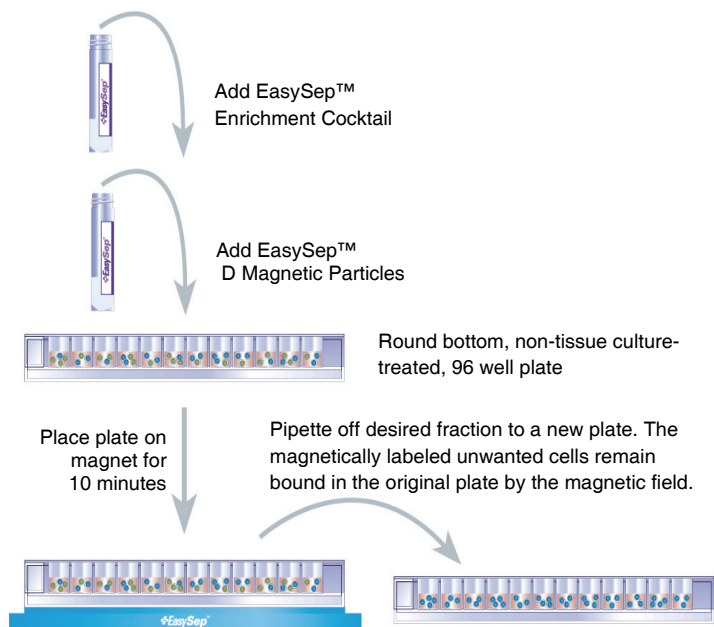
MANUAL EASYSEP™ PROTOCOL USING THE "EASYPLATE" EASYSEP™ MAGNET (CATALOG #18102)

This procedure is used for processing **50 µL - 200 µL** of sample per well (up to 1×10^7 cells per well or 9.6×10^8 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 5×10^7 cells/mL in recommended medium (see Notes and Tips, reverse side). 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™ Human Memory 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 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 **50 µL/mL cells** (e.g. for 200 µL of cells, add 10 µL of particles). Mix well and incubate at room temperature (15 - 25°C) for **5 minutes**.
5. 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.
6. Place the 96 well plate onto the "EasyPlate" EasySep™ Magnet, ensuring that the plate sits securely on the magnet. Incubate for **10 minutes**.
7. 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. 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**FROM WHOLE PERIPHERAL BLOOD**

Prepare a mononuclear cell suspension from whole peripheral blood 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.