

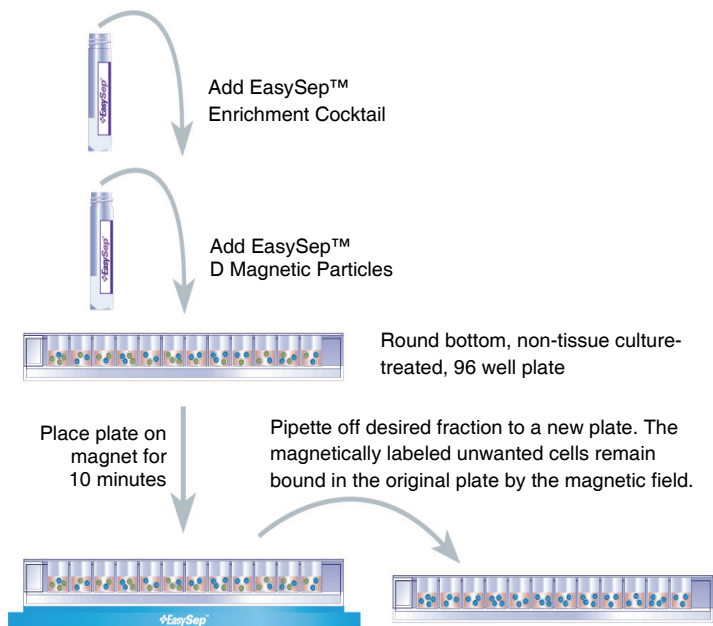
MANUAL EASYSEP™ PROTOCOL USING THE "EASYPLATE" EASYSEP™ MAGNET (CATALOG #18102)

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 (see Notes and Tips, reverse side).
2. Add the EasySep™ Human B 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 **100 µL/mL cells** (e.g. for 200 µL of cells, add 20 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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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. “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.

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 recommended medium. Centrifuge the cells 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 recommended cell concentration, in the recommended medium.