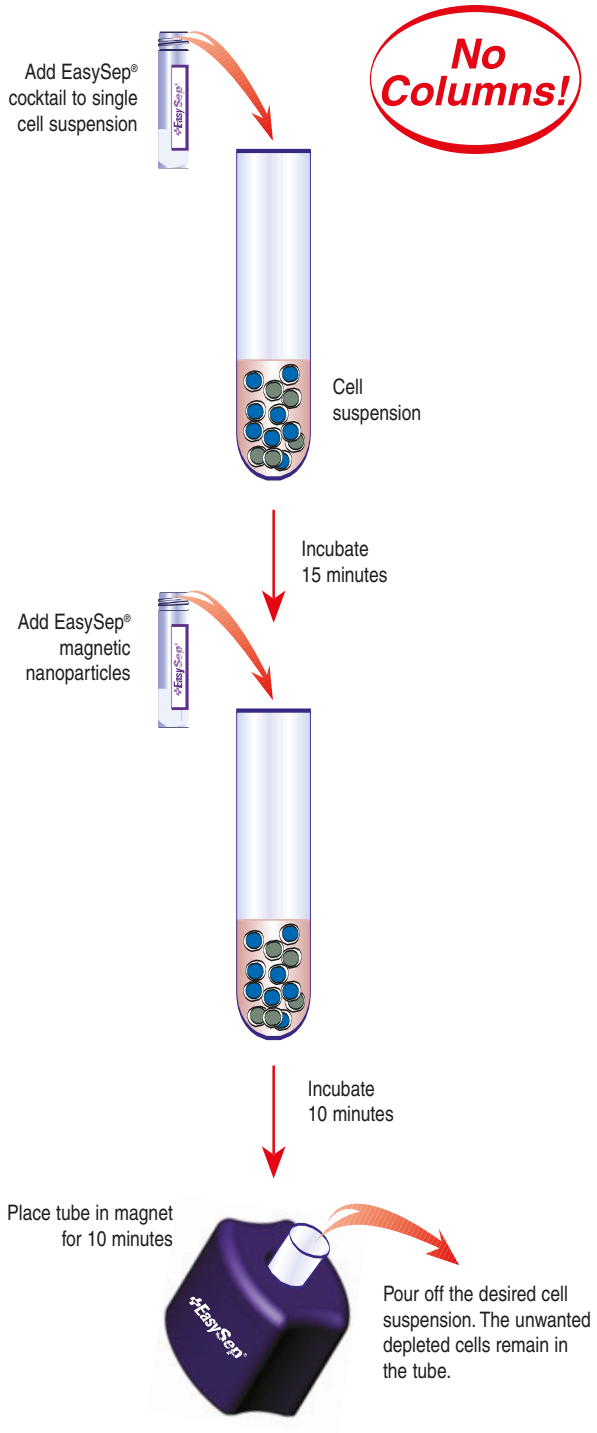
EasySep® Procedure:

Note: This procedure is designed for use with the EasySep® Magnet (18000). If using "The Big Easy" EasySep® Magnet (18001), please refer to www.stemcell.com/technical/18001-PIS.pdf for additional instructions.



PROCEDURE

Positive Selection

EasySep®

Human Cell Depletion Procedure

1. Prepare nucleated cell suspension at a concentration of 1.25 x 10^8 cells/mL in recommended medium (See Notes and Tips). Cells must be placed in a 12 x 75 mm polystyrene tube to properly fit into the EasySep® Magnet. **Do not exceed a volume of 2.0 mL (i.e. 2.5 x 10^8 cells) per tube.** For samples containing 1.25 x 10^7 cells or fewer, resuspend in 100 µL.
   Falcon® 5 mL Polystyrene Round-Bottom Tubes (Becton Dickinson, Catalog #352058) are recommended.
2. Add EasySep® Positive Selection Cocktail at 250 µL/mL cells (eg. for 1 mL of cells add 250 µL of cocktail). Mix well and incubate at room temperature for 15 minutes.
3. Mix EasySep® Magnetic Nanoparticles by pipetting up and down vigorously more than 5 times to ensure that they are in a uniform suspension. Vortexing is not recommended. Add the particles at 125 µL/mL cells (eg. for 1 mL of cells add 125 µL of nanoparticles). Mix well and incubate at room temperature for 10 minutes.
4. Bring the cell suspension to a **total volume** of 2.5 mL by adding recommended medium. Mix the cells in the tube by gently pipetting up and down 2-3 times. Place the tube (without cap) into the magnet. Set aside for **ten** minutes.
5. Pick up the EasySep® Magnet, and in one continuous motion invert the magnet and tube, pouring off the supernatant fraction into a new 12 x 75 mm polystyrene tube. Leave the magnet and tube in inverted position for 2-3 seconds, then return to upright position.
   Do not shake or blot off any drops that may remain hanging from the mouth of the tube. Note: To avoid spilling, the supernatant can be pipetted off to a new 5 mL tube or poured off into a 14 mL tube and then transferred to a 5 mL tube for the second round of separation.
6. Remove the empty tube from the EasySep® Magnet and place the new tube containing the supernatant fraction into the magnet. Set aside for **ten** minutes.
7. Repeat Step 5. The depleted cell suspension is now ready for use.

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