OSUCCC LTB Laboratories Procedure CD138 Positive Selection of Ficolled Mononuclear Cell Fraction from bone marrow or whole blood.			Effective: 11/14/2014
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Version 1.0: (Donna Bucci BS Laboratory Supervisor.)		11/14/2014	4
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1.0 PRINCIPLE

The EasySep® Human CD138 Positive Selection Kit is designed to isolate CD138+ (syndecan-1) cells from fresh or previously frozen bone marrow or peripheral blood mononuclear cells by positive selection. Desired cells are targeted with Tetrameric Antibody Complexes (TACs) recognizing CD138 and dextran-coated magnetic particles. This cocktail also contains an antibody to human Fc receptor to minimize nonspecific binding. Labeled cells are separated using an EasySep® magnet without the use of columns. Cells of interest remain in the tube while unwanted cells are poured off. The CD138 antigen is expressed on normal and malignant plasma cells (but not mature B cells).

2.0 SPECIMEN

Mononuclear cells isolated via Ficoll-paque Plus separation from heparinized bone marrow aspirate or peripheral blood. Any problems or comments regarding sample collection will be noted by the technician.

3.0 MATERIALS AND REAGENTS

1. Purple EasySep Magnet (Catalog #18000)
2. EasySep® Human CD138 Positive Selection Cocktail, 1.0 mL (Catalog# 18357)
3. EasySep® Magnetic Particles, 1.0 ml (Catalog# 18357)
4. Sterile pipets – 5 mL, 10 mL, 25mL, and 50 mL
5. Micropipettes, sterile tips
6. Sterile labeled 50 mL conical tubes, 15 mL conical tubes
7. BD Falcon Polystyrene round bottom tube (Catalog # 352054)
8. Timer
9. Ammonium chloride lysis solution (Catalog# 07800)
10. Ca^{++}/Mg^{++} free PBS + 2% FBS (Catalog# 07905)
11. Wexcide solution and 70% isopropyl alcohol for surface decontamination
12. Benchtop centrifuge
13. Sterile 1.5mL Eppendorf, snap cap tubes
14. Microcentrifuge
15. Plastic storage box (9 x 9 tube format)

4.0 EQUIPMENT

Benchtop centrifuge with adaptors for 50mL and 15mL conical tubes
Microcentrifuge with rotor for 1.5mL Microcentrifuge tubes

5.0 QUALITY CONTROL AND SAFETY

All reagents should be handled using aseptic technique. Containers should be opened only within a biosafety cabinet. All reagents should be observed for signs of bacterial contamination, which includes cloudiness. Isopropyl alcohol is flammable. It is recommended that specimen collection be carried out in accordance with NCCLS document M29T2. No known test sample can offer complete assurance that human blood samples will not transmit infection. Therefore, all derivatives are potentially infectious. Always spray alcohol on the caps before opening solutions. The alcohol can be dried off using gauze. If you have been out of the hood for a while and are wearing the same pair of gloves, use a new pair of gloves. Remember not to touch the sides (inside or outside) of any bottles with your pipette – if you do, dispose of the pipette and start again.

6.0 PROCEDURE

6.0.1. Use a **sterile** environment (i.e. Laminar flow hood) for sample processing.

6.0.2. Perform mononuclear cell separation using current SOP. (T:\HCG\Caliguiri Lab\Procurement\Lab Manual\Protocols\Alliance-OSU\Current SOPs). When total cell count is obtained follow EasySep protocol for CD138 positive selection found in StemCell kit (# 18357). This procedure is used for processing 100uL – 2.5ml of sample (up to 2.5 x 10^8 cells).

6.0.3. Prepare mononuclear cell suspension at a concentration of 1 x 10^8 cells/mL in recommended medium (Ca^{++}/Mg^{++} free PBS + 2% FBS, 1mM EDTA (Catalog# 07950). Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the EasySep magnet. For samples containing fewer the 10^7 cells, very gently resuspend the samples in 100 uL of the medium.

6.0.4. Bone marrow: Add EasySep Positive Selection cocktail at 50uL/mL cells (e.g. for 2mL of cells add 100uL of cocktail; for 100 uL of cells use 5 uL of cocktail).

6.0.5. Mix the sample by flicking the tube, do not vortex or pipet repeatedly. Incubate for 15 minutes at room temperature.

6.0.6. Mix EasySep Magnetic nanoparticles to ensure that they are in a uniform suspension by pipetting up and down vigorously more than 5 times. Vortexing is NOT recommended. Add particles at 50ul/ml cells (e.g. 2 mL of cells – add 100 uL of nanoparticles; for 100 uL of cells use 5 uL of particles). Mix by flicking the tube, do not vortex or pipet repeatedly. Incubate at room temperature for 10 minutes.

6.0.7. Bring the cell suspension to a total volume of 2.5 mL by adding recommended medium with 5 mL pipet. Mix the cells in the tube by gently flicking the tube 2-3 times. Place the tube, without cap into the magnet. Set aside for 5 minutes.

6.0.8. Pick up the EasySep magnet and in on continuous motion invert the magnet and tube, pouring off the supernatant fraction into 50 mL conical tube the magnetically labeled cells will remain inside the tube, held by the magnetic field of the EasySep Magnet. Leave the magnet and tube inverted for 2-3 seconds, and then return the magnet to the upright position. DO NOT SHAKE OR BLOT OFF FOR ANY DROPS THAT MAY REMAIN HANGING FROM THE MOUTH OF THE TUBE.

6.0.9. Remove the tube from the magnet and gently add 2.5 mL of recommended medium with 5 mL pipet. It is important to pipet the medium down the side of the tube and not directly on the sample.

6.0.10. Place the tube back in the magnet and set aside for 5 minutes.

6.0.11. Repeat steps 8 through 10 two more times. For samples with a CD138 starting purity of less than 10-15% additional rounds of separation may be required for optimal purity. Remove the tube from the magnet and resuspend cells in an appropriate amount of desired medium by pipetting down the side of the tube and not directly on the sample. Transfer the sample to a new 15 mL conical tube.

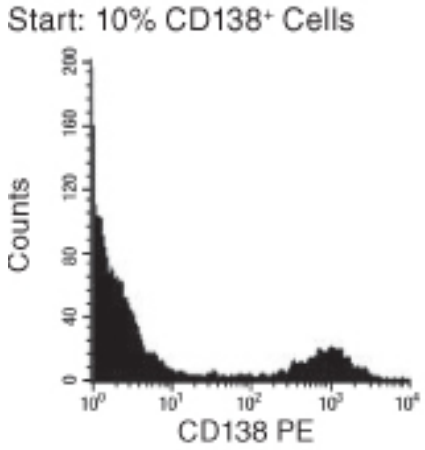
6.0.12. Perform Vi-Cell count according to *OSUCCC LTB_ MNC Separation v.2.3 SOP* on both positive selected fraction and residual cell suspension. Assign a new accession number to and record total cell count for each fraction. Determine how many vials will be banked for each fraction.

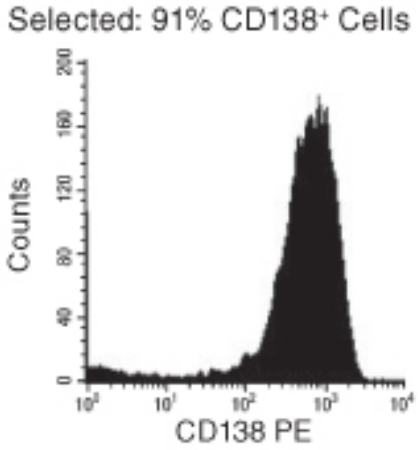
6.0.13. Transfer positive and negative fractions to labeled, sterile Eppendorf tubes, Centrifuge each cell fraction at 1100 rpm for 10 minutes with braking.

6.0.14. Snap freeze pellets in LN_2 and store at -80°C.

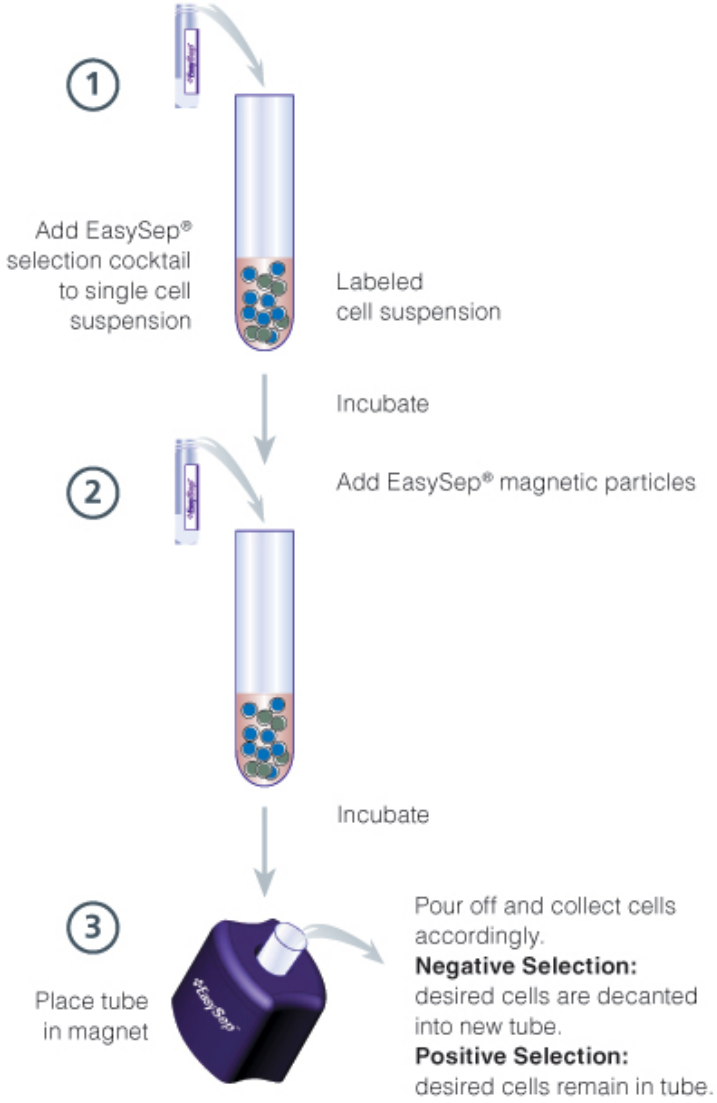
FACS Histogram Results with EasySep® Human CD138 Positive Selection Kit

Starting with Fresh peripheral blood mononuclear cells, the CD138+/Sundecan-1 cell content of the selected cell fraction typically ranges from 85-95%. Please note that purity is highly dependent on the starting sample.





Typical EasySep® Procedure for Human Cells



7.0 LIMITATIONS

Bone marrow or blood cells may be prepared using a red blood cell lysis solution (Ammonium chloride) or by ficoll density separation. For previously frozen mononuclear cells, we recommend incubating the cells with 100ug/ml DNAse 1 for at least 15 minutes at room temperature prior to labeling and separation. Filter clumpy suspensions through. 30 um mesh nylon strainer for optimal results. (Fisher 08-771-1 – BD 40um sterile cell strainer). Dimethylsulfoxide has been shown to prevent formation of ice crystals from forming within mammalian cells when cell suspensions are frozen. Penetration of DMSO into cells is critical to maintain cell viability during cryopreservation and the small size of the DMSO molecular is well-suited to this function. There may be factors related to collection or transportation stress which may decrease the uptake of DMSO into cells thereby inhibiting the cryoprotectant ability of the freezing medium.

8.0 REFERENCES

1. EasySep Human CD138 Selection kit (#18357), StemCell Technologies product brochure #28861. February 2008.