

# Human Mesenchymal Stem cells (Bone Marrow MSC)

## HMSC.BM-100/HMSC.BM-500

### Thawing and Plating Protocol:

Human Mesenchymal Stem Cells (hMSC) are primary cells which can be successfully cultured for approximately five to six passages. The following is the recommended protocol for thawing and subculturing of these cells.

*Note: Once completed media has been formulated, it should be stored at 2-8°C. Avoid extended exposure of the medium to room or higher temperatures. Medium should be equilibrated in a water bath set at 37°C before adding media to any cell culture.*

#### Thawing Cells

1. Remove the vial of cells from dry ice. Defrost the vial of cells in a 37°C water bath with constant, moderate agitation, until ice in the ampoule is no longer visible.
2. Continue to warm the ampoule in the water bath for 30 seconds with gentle agitation.
3. Immediately disinfect the vial with 70% isopropanol.
4. Working in a laminar flow hood, open the vial and transfer the contents to a sterile 15 mL tube.
5. Very slowly, add approximately 10 mL of complete MSC Expansion media (table 1), pre-warmed to 37°C.
6. Centrifuge the suspended cells at 200 x g for 10 minutes.
7. Decant the medium and gently re-suspend the pellet in 10 mL of complete MSC Expansion media (Table 1), then transfer into a T-25 (25cm<sup>2</sup>) culture flask.
8. Observe the cells microscopically to estimate cell viability and then place the flask in an incubator at 37°C, 5% CO<sub>2</sub>, and 90% humidity.
9. Cells will be ready to pass between 3-7 days. Cells should be subcultured at a density of 5,000 to 10,000 cells/cm<sup>2</sup> or desired plating density.

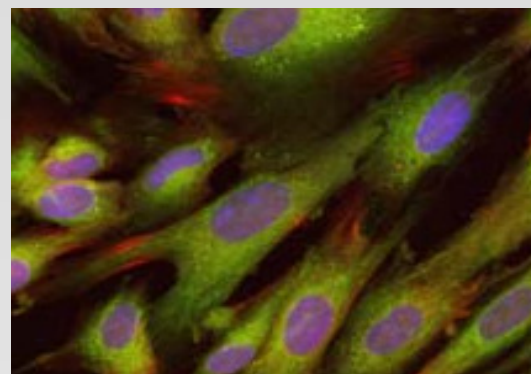


Figure 1: Human Bone Marrow Mesenchymal Stem Cells

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*Note: Antibiotics/ antimycotics should not be used as an alternative to proper aseptic technique.*

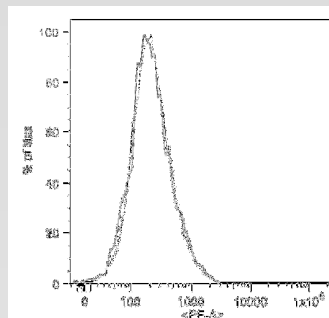
#### Key References:

1. Med J Malaysia. 2008 Jul;63 Suppl A:63-4. The effect of human mesenchymal stem cells on tumour cell proliferation. Sarmadi VH, Heng FS, Ramasamy R.
2. J Clin Neurosci. 2009 Jan;16(1):12-20. Epub 2008 Nov 18. Bone marrow-derived mesenchymal stem cells for the treatment of ischemic stroke. Dharmasaroja P.

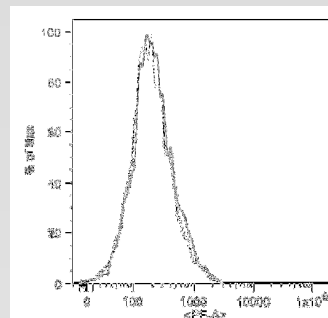
Table 1: Preparation of 500 mL complete hMSC Expansion Media

| Brand | Amount For 500 mL | CET Product                                     | Catalog #                              |
|-------|-------------------|---|--|
| CET   | 450 mL            | CET Human Mesenchymal Stem Cell Expansion Media | HMSC.E.Media-450                       |
| Any   | 50 mL             | Fetal Bovine Serum                              | Refer to Manufacturer's Catalog Number |

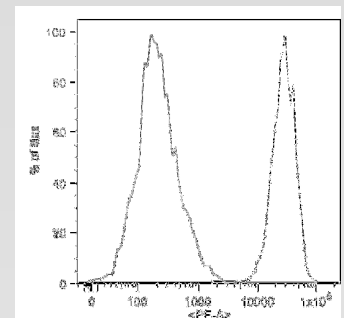
Store at 4°C.



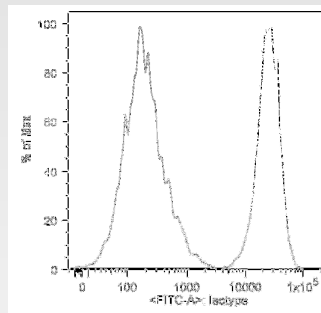
CD14-



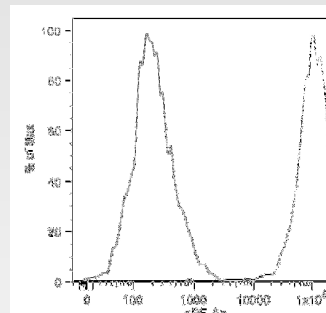
CD45-



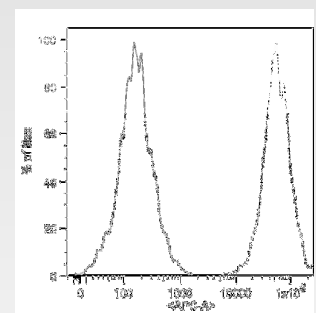
CD29+



CD44+



CD90+



CD105+

### Certificate of Analysis

All hematopoietic, mesenchymal and multipotent stem cells are evaluated by flow cytometry for specific stem cell markers. All other cells are evaluated either by staining, method of isolation or traditional molecular biology techniques. Data is available upon request.

All growth and differentiation media are evaluated by conducting assays to make sure cells either grow or differentiate as indicated on the media label. Data is available upon request.

All cells are tested for HIV-1, HIV-2, Hepatitis B and Hepatitis C using sensitive PCR based assays. All cells test negative for these viruses. However, all human cells must be used in accordance with established laboratory safety procedures and only under the supervision of trained personnel.

All products are for research use only. Not for diagnostic or therapeutic use. CET's products are designed and tested to function with other CET products only. For example, all of our cells are optimized to grow and differentiate in CET media. Although investigators are welcome to formulate their own media, CET cannot and will not guarantee that cells will function as indicated in the product brochure. Moreover, such third party use will void CET's obligation to replace cells, should they not function as indicated.

