Mesenchymal Stem Cell/Multipotent Unrestricted Somatic Stem Cell Expansion Media
HMSC.E.Media-450/ HMpC.E.Media-450

Media Usage Protocol:

Mesenchymal Stem Cell/Multipotent Unrestricted Somatic Stem Cell Expansion Media
is designed to be used with Human Adipose Derived Mesenchymal Stem Cells, Human Amniotic Membrane Mesenchymal Stem Cells, Human Bone Marrow Mesenchymal Stem Cells, Human Wharton's Jelly Mesenchymal Stem Cells and Human Multipotent Unrestricted Somatic Stem Cells, all of which are available separately. When used as directed, this media will support robust growth and expansion of these stem cells for approximately 10 passages. We do not recommend passing cells any further since they tend to show a dimunition in differentiation potential and a slowdown in growth rate. The following is the recommended protocol for the usage of this media.

Note: Once complete media has been formulated, it should be stored at 4°C. Avoid extended exposure of the media to room or higher temperatures. Media should be equilibrated in a water bath set at 37°C before adding to any cell culture.

Figure 1: Mesenchymal Stem Cell / Multipotent Unrestricted Somatic Stem Cell Expansion Media

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

Additional Reagents Needed
1. Fetal Bovine Serum, High Grade or Characterized. Store in aliquots of 50mL at -20°C.
2. Penicillin/Streptomycin/Amphotericin B solution, 100X or Penicillin/Streptomycin solution, 100X. These solutions should be portioned in 5mL aliquots, stored at -20°C and never freeze/thawed. Although antimycotics are not absolutely necessary, CET highly recommends their usage for long term cell culture.

Formulating Complete Mesenchymal Stem Cell/Multipotent Unrestricted Somatic Stem Cell Expansion Media
1. Defrost 50mL of fetal bovine serum and 5mL of antibiotic/antimycotic solution in a 37°C water bath until ice in the tubes is no longer visible.
2. Immediately disinfect the tubes and the bottle containing the base media with 70% isopropranol.
3. Working in a laminar flow hood, remove 5mL of the media from the bottle and discard. This and all other procedures must be done in a sterile manner.
4. Add 50mL of the fetal bovine serum to the base media.
5. Add 5mL of the antibiotic/antimycotic solution to the base media.
6. Cap the bottle containing the now complete media and gently swirl a few times. The complete media is now ready to use.

Thawing Mesenchymal Stem Cells/Multipotent Unrestricted Somatic Stem Cells and Using the Media
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 15mL tube.
5. Very slowly, add approximately 10mL of complete Mesenchymal Stem Cell/Multipotent Unrestricted Somatic Stem Cell Expansion media, pre-warmed to 37°C.
6. Centrifuge the suspended cells at 200 x g for 10 minutes.
7. Decant the medium and gently resuspend the pellet in 10mL of complete Mesenchymal Stem Cell/Multipotent Unrestricted Somatic Stem Cell Expansion media, then transfer into a T-25 (25cm) culture flask.
8. Observe the cells microscopically to estimate cell viability and then place the flask in an incubator at 37°C, 5% CO_2 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 or desired plating density.

Key References:
1. Methods Mol Biol. 2008;449:59-67.
Adipose-derived stem cells.
Fraser JK, Zhu M, Wulur I, Alfonso Z.

2. Tissue Eng Part A. 2009 Jan 12.
Growth, Metabolism, and Growth Inhibitors of Mesenchymal Stem Cells.
Schop D, Janssen FW, van Rijn LD, Fernandes H, Bloem RM, de Bruijn JD, van Dijkhuizen-Radersma R.

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.

Table 1: Preparation of 500 mL complete Mesenchymal Stem Cell/ Multipotent Unrestricted Somatic Stem Cell Expansion Media

Brand	Amount For 500 mL	CET Product	Catalog #
CET	450 mL	CET Mesenchymal Stem Cell/ Multipotent Unrestricted Somatic Stem cell Expansion Media	HMSC.E.Media-450/ HMpC.E.Media-450
Any	50 mL	Fetal Bovine Serum	Refer to Manufacturer's Catalog Number

Store at 4°C.