

Human Mesenchymal Stem Cells (Amniotic Membrane MSC) HMSC.AM-100/HMSC.AM-500. Store cells at -80°C or in liquid nitrogen.

Thawing and Plating Protocol:

Human mesenchymal stem cells (hMSC) are primary cells which can be cultured for approximately five to six passages. Amniotic membrane MSC are isolated from the amniotic membrane after the membrane has been stripped of amniotic epithelial cells. 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 resuspend the pellet in 10 mL of complete MSC Expansion Media (Table 1), then transfer into a T-25 (25 cm²) culture flask.
8. Observe the cells microscopically to estimate cell viability and then place the flask in an incubator at 37°C, 5% CO₂ and 90% humidity.
9. Cells will be ready to be passed between 3-7 days. Cells should be subcultured at a density of 5,000 to 10,000 cells/cm² or desired plating density.

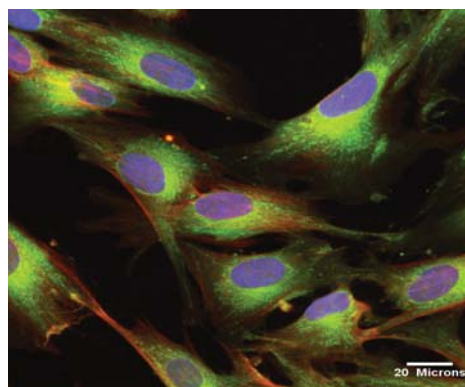


Figure 1: Human Amniotic Membrane Mesenchymal Stem Cells
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Note: Antibiotics/Antimycotics should not be used as an alternative to proper aseptic technique.

Key References:

1. Adv. Biochem. Eng. Biotech. 2010; March 17. Epub. Alternative Sources of Adult Stem Cells: Amniotic Membrane. Wolbank S, Van Griensven M, et al.
2. Cells Tissues Organs. 2010; March 9. Epub. Isolation of Mesenchymal Stem Cells with Neurogenic Potential from the Mesoderm of the Amniotic Membrane. Chang YJ, Hwang SM, Tseng CP, et al.

Table 1: Preparation of 500 mL complete hMSC Expansion Media

Brand	Amount for 500 mL	CET Product	Catalog Number
CET	450 mL	CET Human Mesenchymal Stem Cell Expansion Media	HMSC.E.Media-450
Any	50 mL	Fetal Bovine Serum	Refer to Manufacturers' Catalog Number

Store Media at 4°C.

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.

Disclaimer

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.

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