Human Amniotic Epithelial Stem Cells
HAEC-100/HAEC-500

Thawing and Plating Protocol:

Human Amniotic Epithelial Stem Cells (hAE) are primary cells isolated from the surface membrane of fresh placentas. HAE cells retain embryonic stem cell like characteristics and express Nanog, Oct-4 and Sox-2. These cells are slow growing and can only be cultured for one or two subsequent passes. They should be cultured at high-density using a coated tissue culture dish. 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.

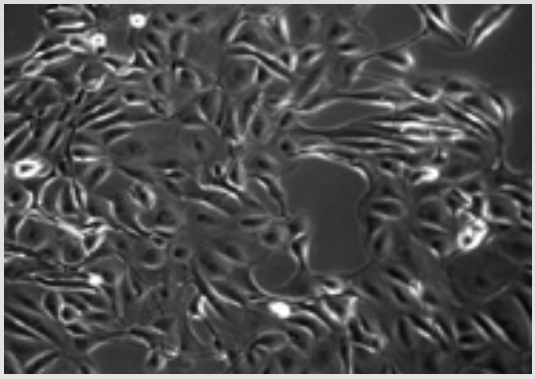

Figure 1: Human Amniotic Epithelial Stem Cells

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

Items Needed Before Thawing the Cells

1. You will need 1 T-25 tissue culture dish, pre-coated with either Type I or Type IV collagen. We recommend using either Becton Dickinson or Sigma Aldrich as the manufacturer. Please follow manufacturer's direction and have the plate pre-coated and ready before cells are thawed.

Thawing Cells

1. Remove the vial of cells from dry ice. Defrost the vial of cells in a 37℃ water bath until ice in the ampoule is no longer visible.
2. Immediately disinfect the vial with 70% isopropanol.
3. Working in a laminar flow hood, open the vial and transfer the contents to a sterile 15 mL tube.
4. Very slowly, add approximately 10 mL of complete Amniotic Epithelial Stem Cell Expansion Media (Table 1) pre-warmed to 37℃.
5. Centrifuge the suspended cells at 200 x g for 10 minutes.
6. Decant the medium and gently re-suspend the pellet in 10 mL of complete Amniotic Epithelial Stem Cell Expansion Media (Table 1), then transfer into a coated T-25 (25 cm^2) culture flask.
7. Observe the cells microscopically to estimate cell viability and place the flask in an incubator at 37℃, 5% CO_2 and 90% humidity.
8. Cells will be ready to pass in approximately 14 days and media should be replaced every 3 days. Cells have limited subculturing potential and are best used at the first passage or at the latest, the next passage.

Table 1. Preparation of 450 mL complete Amniotic Epithelial Stem Cells Expansion Media

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

Store at 4°C.

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Key References:

1. Biol Reprod. 2007 May 9. Epub.
Stem Cells Derived from Human Fetal Membranes Display Multi-Lineage Differentiation Potential.
Ilancheran S, Michalska A, Peh G, Wallace EM, Pera M, Manuelpillai U.

2. Chin Med J (Engl). 2006 Dec 20;119(24):2101-7.
Transplantation of human amniotic epithelial cells improves hindlimb function in rats with spinal cord injury.
Wu ZY, Hui GZ, Lu Y, Wu X, Guo LH.

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.

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