Frequently Asked Questions About Adipose Stromal Cell Cultures

1. How are adipose stromal cells (ASCs) shipped?

The ASC cultures are in a glass ampoule that is shipped on dry ice.

2. What is the composition of ASC growth medium?

For ASC growth medium, use the following components:

- 0.5% FBS (human ASC) or 2% FBS (mouse ASC)
- 1X ITS-X (insulin, transferrin, selenium with ethanolamine, Invitrogen #51500, 100X)
- 0.2% bovine serum albumin (BSA)
- 1X linoleic acid (Sigma #L9530,100X)
- 100 μM L-ascorbate-2-phosphate
- 100 μM 55 mM β–mercaptoethanol
- 10 ng/ml EGF (human or mouse EGF based on the species)
- 5 ng/ml PDGF-BB (human or mouse PDGF based on the species)
- 1 nM dexamethasone
- 60:20:20 ratio of DMEM:MCDB 201:MCDB 131
  NOTE: MCDB 201(Sigma #M6770) and MCDB 131 (Invitrogen 10372-019) are media formulations.

3. How can the ASC be differentiated toward the adipogenic lineage?

- Culture cells till they reach 60-70% confluence.
- Replace ASC growth medium with differentiation medium. For human cells, use Stempro® Adipogenic Differentiation kit basal medium and supplement (Invitrogen # A1007001). For murine ASC, induce for three days with DMEM/HF12 (1:1)/10%FBS medium containing 2.5 μg/ml insulin, 500 μM IBMX, and 50 μM dexamethasone. Then replace the induction medium with differentiation medium consisting of growth medium with 2.5 μg/ml insulin and 50 μM dexamethasone.
- Replace the differentiation medium every three days for 14-21 days during which time lipid vacuoles will form.
- If desired, fix the cells for lipid staining e.g. with Oil Red O and hematoxyllin counterstain.

4. What is the maximum recommended number of passages before differentiation capacity is compromised?

In general, the differentiation capacity of ASC begins to decline at around passage 6-10. It is advisable to perform differentiation at early passages 2-4. Differentiation capacity is negatively affected by allowing the cells to become confluent. Thus, be sure to subculture ASC before they reach confluence, preferably by 80%.

5. How should ASC be cryopreserved and stored?

- Prepare ~ 500,000 cells per cryovial in ASC freezing medium. ASC freezing medium is composed of ASC growth medium supplemented with 10% FBS and 10% DMSO.
- Cells resuspended in ASC freezing medium should be immediately placed in a controlled rate freeze machine that reduces the temperature at a controlled rate of -1°C/min. Alternatively, ASC in plastic cryovials can be placed in an ethanol bath at -80°C overnight before being placed in liquid nitrogen vapor.
- It is advisable to store the cell lines in liquid nitrogen (glass ampoules) or liquid nitrogen vapor (plastic cryovials) for long term storage.
- Cells stored in freezing medium should retain their viability indefinitely.

6. How should cryopreserved ASC be thawed?

- Prepare growth medium (see Coriell Shipping Sheets for cell line-specific information).
- Remove one ampoule or cryovial from frozen storage and place immediately in a 37°C water bath and agitate vigorously.
- Once completely thawed, wipe ampoule or cryovial with a 70% alcohol pad. Score the neck of a glass ampule and open utilizing an ampule opener.
- Remove the contents of the ampoule or cryovial using a sterile pipette and place in a 15 ml centrifuge tube containing 3 ml of growth medium..
- In order to remove traces of the cryoprotectant, centrifuge the tube for 5 min at 60-100xg at room temperature.
- Remove the supernatant, resuspend the cell pellet, and transfer the cell suspension to a T25 flask with a final volume of 5 ml of ASC growth medium.
- If a cell count is required, mix the contents of the flask gently with a 1 ml pipette and remove 0.2 ml for trypan blue staining and cell counting.
- Place the flask with the remaining cells in a 37°C incubator. Gently swirl the flask to evenly distribute the cell suspension.
- Incubate the ASC at 37°C, 5% $C0_2$, and ambient air.

  NOTE: mouse ASC grow better in 3% 0_2.

- Replace the ASC growth medium the day after seeding.
- Subculture ASC by 80% confluency.

NOTE: Differentiation capacity is negatively affected by allowing the cells to become confluent. Thus, be sure to subculture ASC before they reach confluence.