1. **What growth medium is used to culture amniotic fluid-derived cell lines?**
   AmnioMAX C-100 Basal Medium (Life Technologies #17001-074)
   AmnioMAX C-100 Supplement (Life Technologies # 12556-023)
   Add 75 ml bottle of AmnioMAX C-100 supplement to the 450 ml bottle of basal medium. This complete medium can be used for up to 2 weeks, provided medium is kept refrigerated.

2. **What other reagents are needed?**
   0.53 mM EDTA in HBSS
   0.04% trypsin/0.53 mM EDTA in HBSS

3. **Should amniotic fluid-derived cell lines be cultured using a substrate?**
   Yes, culture amniotic fluid-derived cell lines on 0.1% Gelatin (Sigma-Aldrich #G-2500) coated cultureware.

4. **How is an amniotic fluid-derived cell line subcultured?**
   Volumes are for 25-cm^2 flask
   NOTE: It is important to subculture the cells when they are sub-confluent or just confluent, as they lose viability when kept at confluence.

   1. Remove medium by aspiration.
   2. Add 3 ml EDTA.
   3. Observe monolayer under inverted scope until cells begin to separate.
   4. Remove EDTA.
   5. Add 3 ml Trypsin-EDTA, and incubate at 37°C for 5-7 minutes.
   6. After most of the cells come loose (with gentle tapping of the flask), add at least 3 ml of growth medium.
   7. Break up cell clumps by gentle trituration.
   8. Transfer cell suspension to a centrifuge tube.
   9. Remove aliquot for cell count.
   10. Centrifuge recovered cell suspension at 60-100 x g for 10 minutes at 8-10°C.
   11. Aspirate supernatant and re-suspend cell pellet in growth medium.
   12. Inoculate flasks with 2.0 to 3.5 x 10^5 cells in ~5 ml growth medium per T25 flask.
   13. Re-feed original flask with 5 ml growth medium as a back-up culture.
   14. Change medium every 3-5 days until the cell sheet is ~75% confluent.

5. **What is the freezing medium used to cryopreserve amniotic fluid-derived cell lines?**
   Growth medium + 15% FBS + 5% DMSO

6. **How should amniotic fluid-derived cell lines be cryopreserved and stored?**
   1. Place cells into a single-cell suspension, count and pellet as indicated in the subculture protocol above.
   2. Resuspend the cells in freezing medium to a seeding density of 5.0e5 viable cells per ml
   3. Aliquot 1 ml into each cryovial or ampule.

4. Cells resuspended in freezing medium should be immediately placed in a controlled rate freeze machine that reduces temperature at a controlled rate of -1°C/min. Alternatively, cryovials can be placed in an ethanol bath at -80°C overnight before being placed in liquid nitrogen vapor.
5. Frozen cell stocks are stored in liquid nitrogen tanks. Glass ampules are submerged in liquid, plastic cryovials are stored in vapor phase.

*Suppliers of reagents are listed for the convenience of culture recipients only. Such lists are not intended to be either selective or exhaustive, and Coriell Institute does not recommend specific products or suppliers. Other media and reagents may be satisfactory, but have not been tested.