Frequently Asked Questions About Melanocyte Cell Cultures

1. What growth medium is used to culture human melanocytes?

   The preferred melanocyte medium is MGM-4 Bulletkit. This medium includes growth factors and is available from Lonza (catalog #CC-3249). An alternative growth medium is Medium 254 available from Invitrogen (#M254500) which is supplemented with HMGS (human melanocyte growth supplement, #S0025).

2. What is the freezing medium used to cryopreserve human melanocytes?

   Ham's F12 + 10% FBS + 10% DMSO.

3. Is it possible to use the melanocyte growth medium for freezing the cells?

   It is possible to freeze the cells in growth medium supplemented with 10% DMSO.

4. What protocol should be used for freezing melanocytes?

   To freeze melanocytes, Versene (0.2 g/L EDTA•4Na in phosphate-buffered saline, Invitrogen 15040), 0.05% trypsin/0.53 mM EDTA in HBSS, soybean trypsin inhibitor (STI, 2 mg/ml), melanocyte growth medium, and 2x melanocyte freeze medium (Ham's F12, 20% FBS, and 20% DMSO) are needed for the following procedure for T75 flasks of confluent cells:

   1. Prepare the 2x freeze medium and keep it refrigerated or on ice until use.
   2. Each flask that is to be pooled for the freeze (freeze pool) should be examined microscopically for contamination and any unusual growth pattern.
   3. Aspirate the growth medium from each flask. Wash the cells with 7 ml D-PBS then rinse with Versene solution (maximum 5 minutes at room temperature). Trypsinize the cells with 2.5 ml 0.05% trypsin/0.53 mM EDTA.
   4. Examine the flasks microscopically to make sure the cells have rounded up - typically within 1-3 minutes.
   5. Once the cells have lifted, add 10 ml STI to each flask to inactivate the trypsin. Gently triturate and then transfer the cell suspension from all flasks. Pool the cells.
   6. Remove an aliquot of the freeze pool, count the cells, and calculate the total viable cells.
   7. Centrifuge the cells at 60-100 x g for 10 minutes at 8-10°C.
   8. Using culture medium without serum or DMSO, resuspend the cell pellets in half the volume needed for a cell density of 500,000 cells/ml.
   9. For the second half of the volume, add the 2X freezing medium slowly (drop by drop) while gently mixing the cells.
   10. Dispense 1 mL of the cell suspension to each glass ampule or plastic cryovial.

11. Freeze the ampules or cryovials at a rate of 1°C per minute.
12. Frozen cell stocks are stored in liquid nitrogen tanks. Glass ampules are submerged in liquid. Plastic cryovials are stored in the vapor phase.

5. Is it necessary to grow melanocytes on collagen-coated culture vessels? If so, what supplier is recommended?

Extensive testing has shown that collagen IV (Sigma C7521) at a concentration of 0.67 $\mu g/cm^2$ is optimal for enhancing plating efficiency of melanocytes.