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Melanoma Dissociated Tumor Cells Thawing and Culturing Procedure

Safety:

- **Primary tumor cells should be contained in a Class II biological safety cabinet and handled using Biosafety Level 2 (BSL-2) work practices and facilities. (1)**

Cell Culture Media and Antibiotics:

- **Recommended cell culture media (3):**
  - DMEM-F12 medium with HEPES and L-glutamine (Lonza 12-719 F)
- **Antibiotics:**
  - Penicillin (100 IU/mL)
  - Streptomycin (100 µg/mL)
  - Fungizone (2 µg/mL)
- **Supplements:**
  - B-27 Supplement, serum-free (ThermoFisher Sci 17504-044)
  - 20 ng/ml EGF
  - 10 ng/ml bFGF
  - 10 µg/mL insulin (activates the Akt pathway; see reference 3)
- **Supplements recommended for unthawing samples**
  - DNase I (1 mg/mL), resuspended in PBS without calcium or magnesium and filter-sterilized

Unthawing Procedure:

1. On the day of the thaw, pre-warm 19 mLs of recommended cell culture media in a 37°C incubator.
2. Divide the media into two 15-mL conical tubes (1 tube with 9 mLs of media; 1 tube with 10 mLs of media).
3. Quickly thaw the DTC sample in a 37-40°C water bath until 2mm crystals remain. Move sample through the water to speed thawing.

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4. Slowly add the 1-mL DTC sample to 9 mLs of pre-warmed media. Gently mix by inversion (do not vortex).
5. Centrifuge the conical tube at 300 x g for 5-10 minutes (no brake). Carefully remove the supernatant.
6. After the initial wash to remove DMSO from the sample, gently resuspend the pellet in the remaining 10 mLs of pre-warmed cell culture media.
7. If the cells clump together, perform the following:
    a. Add DNase I (1 mg/mL) to the cell culture media.
    b. Incubate at 37°C for 5-10 minutes to facilitate digestion of released DNA.
    c. Centrifuge the conical tube at 300 x g for 5 - 10 minutes (no brake).
    d. Carefully remove the supernatant containing DNase I.
    e. Gently suspend the pellet in 10 mLs of pre-warmed cell culture media. Go to the next step.
8. Allow cells to rest for at least 1 hour in the media at 37°C before checking the viability and plating.
9. Check the viability and cell number of the sample. (4)
10. Plate the cells according to assay requirements.

Culturing Primary Cells:

1. For sphere formation assays, poly-HEMA (Sigma Aldrich) or Hydrogel-coated plates or dishes are recommended (5). Melanospheres can also be maintained in non-adherent flasks (2).
2. The media recipe listed above can be used as a base media for tissue culture.
3. Additional supplementation with heparin may be required (1 ng/ml up to 4 µg/ml; see references 2 and 5, depending on the requirements of the assay.
4. Please see the literature for further supplementation recommendations required by the cell types targeted for *in vitro* expansion.

References:

1. Centers for Disease Control and Prevention. 2009. Biosafety in Microbiological and Biomedical Laboratories (BMBL). U.S. Dept. of Health and Human Services. 5th ed.
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3. Chi, M.; Y. Ye; X. D. Zhang; J. Chen. 2014. Insulin induces drug resistance in melanoma through activation of the PI3K/Akt pathway. *Drug Des Devel Ther* 8:255-262.
4. Chan, L. L.; D. J. Laverty; T. Smith; P. Nejad; H. Hei; R. Gandhi; D. Kuksin; J. Qiu. 2013. Accurate measurement of peripheral blood mononuclear cell concentration using image cytometry to eliminate RBC-induced counting error. *J Immunol Methods.* 388:25-32.
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