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Colorectal Cancer 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 (2):**
    - DMEM-F12 medium with 10% fetal bovine serum and 10% L-glutamine
- **Antibiotics:**
    - Penicillin (500 IU/mL)
    - Streptomycin (500 µg/mL)
    - Gentamicin (100 µg/mL)
    - Amphotericin B/Fungizone (12.5 µg/mL)
    - Metronidazole (5 µg/mL)
- **Supplements:**
    - 8 ng/mL bFGF
- **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.

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 (3).
10. Plate the cells according to assay requirements.

Culturing Primary Cells:

1. For sphere formation assays, a serum-free media may also be used (such as StemPro hESC SFM, ThermoFisher Scientific). Cells can be plated on ultra-low attachment tissue culture flasks (4).
2. The media recipe listed above can be used as a base media for tissue culture. Antibiotics should be added to the tissue culture media and any wash buffers to prevent microbial growth.
3. Please see the literature for further supplementation recommendations required by the cell types targeted for *in vitro* expansion.

References:

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3. 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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