**Melanoma Dissociated Tumor Cells**

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**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 (2).

**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)
  + 10 ng/ml bFGF
  + 20 ng/ml EGF
  + 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 Instructions**

* On the day of the thaw, pre-warm 19 mls of recommended cell culture media in a 37°C incubator.
* Divide the media into two 15-ml conical tubes (1 tube with 9 mls of media; 1 tube with 10 mls of media).
* Quickly thaw the DTC sample in a 37-40°C water bath until 2mm crystals remain. Move sample through the water to speed thawing.
* Slowly add the 1-ml DTC sample to 9 mls of pre-warmed media. Gently mix by inversion (do not vortex).
* Centrifuge the conical tube at 300 x g for 5-10 minutes (no brake). Carefully remove the supernatant.
* 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.
* If the cells clump together, perform the following:
  + Add DNase I (1 mg/ml) to the cell culture media.
  + Incubate at 37°C for 5-10 minutes to facilitate digestion of released DNA.
  + Centrifuge the conical tube at 300 x g for 5 - 10 minutes (no brake).
  + Carefully remove the supernatant containing DNase I.
  + Gently suspend the pellet in 10 mls of pre-warmed cell culture media. Go to the next step.
* Allow cells to rest for at least 1 hour in the media at 37°C before checking the viability and plating.
* Check the viability and cell number of the sample (4).
* Plate the cells according to assay requirements.

**Culturing Primary Cells**

* For sphere formation assays, poly-HEMA (Sigma Aldrich) or Hydrogel-coated plates or dishes are recommended (5). Melanospheres can also be maintained in nonadherent flasks (2).
* The media recipe listed above can be used as a base media for tissue culture.
* 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.
* Please see the literature for further supplementation recommendations required by the cell types targeted for *in vitro* expansion.

**References**

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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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**Thawing and Culturing Procedures**