**Melanoma Dissociated Tumor Cells**

 **www.conversantbio.com**

**601 Genome Way, Northwest, Huntsville, AL 35805 Tel: (866) 838- 2798**

Contents are for research use only.

Version 02-2016

**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**

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.
2. Czyz, M.; K. Koprowska; M. Sztiller-Sikorska. 2013. Parthenolide reduces the frequency of ABCB5-positive cells and clonogenic capacity of melanoma cells from anchorage independent melanospheres. *Cancer Biol Ther* 14:135-145.
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.
5. Mukherjee, N.; S. N. Reuland; Y. Lu; Y. Luo; K. Lambert; M. Fujita; W. A. Robinson; S. E. Robinson; D. A. Norris; Y. G. Shellman. 2015. Combining a BCL2 inhibitor with the retinoid derivative fenretinide targets melanoma cells including melanoma initiating cells. *J Invest Dermatol.* 135:842-850.

**Thawing and Culturing Procedures**