DISCOVERY
LIFE SCIENCES

Science at your Service.™

Ovarian Dissociated Tumor Cell 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. (2)**

Cell Culture Media and Antibiotics:

- **The presence or absence of serum can influence ovarian tumor cells, causing either differentiation or retention of cancer stem cell (CSC)-like properties in culture (2). Two base media formulations are described below. Please see the literature for assay-specific requirements.**

- **Recommended base media for differentiated ovarian DTCs (2):**
  - DMEM/F12, GlutaMax (ThermoFisher Scientific)
  - 20% fetal bovine serum (FBS)
- **Recommended base media for CSC ovarian DTCs (2,3):**
  - DMEM/F12 or MEBM media (Lonza)
  - Insulin (5 µg/mL)
  - Human recombinant epidermal growth factor (EGF; 10 ng/mL)
  - Basic fibroblast growth factor (LIF; 12 ng/mL)
  - Bovine serum albumin (BSA; 0.3%)
- **Antibiotics (4):**
  - 2Penicillin (100 IU/mL)
  - Streptomucin (100 µg/mL)
  - Fungizone (2 µg/mL)
- **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.

Ovarian Dissociated Tumor Cell Thawing and Culturing Procedure - Continued

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 (5).
10. Plate the cells according to assay requirements.

Culturing Primary Cells:

1. The media recipe listed above can be used as a base media for tissue culture (2, 3).
2. 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. Liu, T.; W. Cheng; D. Lai; Y. Huang; L. Guo. 2010. Characterization of primary ovarian cancer cells in different culture systems. *Oncol Rep.* 23:1277-1284.
3. Silva, I. A.; S. Bai; K. McLean; K. Yang; K. Griffith; D. Thomas; C. Ginestier; C. Johnston; A. Kueck; R. K. Reynolds; M. S. Wicha; R. J. Buckanovich. 2011. Aldehyde dehydrogenase in combination with CD133 defines angiogenic ovarian cancer stem cells that portend poor patient survival. *Cancer Res.* 71:3991-4001.
4. Hogg, S. J.; J. J. Evans; P. H. Sykes; K. Chitcholtan. 2015. A method to investigate the anti-metabolic activity of anti-cancer agents on ovarian cancer cells cultured in a 96-well high throughput format. *J. Ovarian Res.* 8:43.
5. 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.