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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**

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 (bFGF; 10 ng/ml)
	+ Leukemia inhibitory factor (LIF; 12 ng/ml)
	+ Bovine serum albumin (BSA; 0.3%)
* Antibiotics (4):
	+ Penicillin (100 IU/ml)
	+ Streptomycin (100 μg/ml)
	+ Fungizone (2 μg/ml)
* Supplements recommended for thawing 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 (5).
* Plate the cells according to assay requirements.

**Culturing Primary Cells**

* The media recipes listed above can be used as a base media for tissue culture (2, 3).
* Please see the literature for further supplementation recommendations required by the cell types targeted for *in vitro* expansion.

**References**

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**Thawing and Culturing Procedures**

**Ovarian Dissociated Tumor Cells**