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 L-glutamine
  - 5 mM HEPES
  - 0.1% sodium bicarbonate
  - 0.4% BSA or 10% FBS
- Antibiotics
  - Normocin (Invivogen; 100 µg/mL)
- Supplements
  - 20 ng/ml EGF
  - 10 ng/ml bFGF
  - 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 supernatant containing DNase I.
  - Gently resuspend the pellet in 10 mLs of pre-warmed cell culture media. Go to 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.
- Plate the cells according to assay requirements.

Culturing Primary Cells
- For sphere formation assays, using untreated tissue culture flasks is recommended to reduce cell adherence (4).
- The media recipe listed above is a base media for thawing and acclimating lung tumor cells, in preparation for cell culture.
- Additional supplementation will be required to expand lung tumor primary cells in vitro (3), depending on the end-goal of the assay. Please see the literature for supplementation recommendations, depending on the specific needs of the cell types targeted for expansion.

References
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