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FL-HCC Spheroid Freezing Medium

Used to Freeze Spheroids From

Human Fibrolamellar Hepatocellular Carcinoma (FL-HCC) Spheroids

FL-HCC Spheroid Freezing Medium contains DMSO and is optimized for the cryopreservation of FL-HCC spheroids.

I. Required Supplies

1. FL-HCC Spheroid Freezing Medium (Catalog # 35201-050)

II. Safety Working with Human Cancer Cells

1. Assume that all cells isolated from human tissue are potentially hazardous even though they test negative for human pathogens
2. Use care in handling human cancer cells by preparing a 10% bleach solution and dip pipettes, pipette tips and plasticware into the bleach solution before placing in biohazard bag.
3. Always wear personal protective equipment
    a. Wear appropriate lab coat, gloves and safety glasses
4. All operations should be conducted using aseptic technique within a biosafety cabinet

III. Cryopreserving Human FL-HCC Spheroids and Unsettled Cells

1. The day before cryopreserving place Spheroid Freezing Media in refrigerator to thaw.
2. When ready to cryopreserve the spheroids, remove the dishes/flasks from the incubator and transfer to the safety cabinet.
3. Using a pipette, remove the spheroid suspensions and transfer into conical tube. (Treat the cells in the unsettled dish/flask as spheroids so you collect the new spheroids)
4. Spheroid suspension from multiple dishes/flasks can be pooled into 50ml conical tubes.
5. Rinse each plate with media and add to the tube of spheroids.
6. Ensure there is a homogenous suspension then take an aliquot for counting.
7. Follow counting steps above in section V. 14-16.
8. Transfer tubes of spheroids into incubator to allow spheroids to settle for up to 2 hours.
9. Once spheroids have settled remove the media above the spheroids leaving 0.5-1ml at the bottom of the tube.
10. Transfer the unsettled suspension into another conical tube. You may pool the unsettled suspension from multiple dishes/flasks.
11. Pellet the unsettled suspension at 1500 RPM for 5 minutes.

12. Remove the supernatant taking care not to disturb the pellet.
13. Resuspend the pellet in enough Spheroid Freezing Media to freeze 1-2 million cells per vial.
14. Transfer the unsettled cells in freezing media into the tube of spheroids and gently resuspend into homogenous suspension
15. Transfer 1ml cryo spheroid suspension per tube to freeze.
16. Slow freeze in Styrofoam container in -80° ultralow freezer or in stepdown freezer
17. Store in liquid nitrogen freezer for long term storage.

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