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INSTRUCTIONS FOR THAWING AND PLATING OF CELLS

Cell viability is affected by the efficiency with which you perform certain processes. Cells are negatively affected by temperature fluctuations — severe damage to membrane and cytoskeletal components will result. Upon receiving shipment of the cell vial(s), promptly remove the vial(s) from the dry ice shipping container and immediately transfer to storage under liquid nitrogen, where cells should remain until ready for thawing. Review your thawing and plating process before initiation and move quickly and with purpose when working with these cells.

Materials Required for Thawing and Plating Cells:

- Vial of cryopreserved cells, kept properly stored under liquid N_2 until the moment this protocol is initiated
- Cell Systems growth medium (appropriately supplemented with CultureBoost™ or RocketFuel™)
- 1 vial of CultureBoost™ for resuspending cells (included with order)
- Attachment Factor™, 5mL per T-75 culture flask
- Suitable culture vessel, e.g. T-75 culture flask
- Sterile lab gloves
- Cell culture incubator (37°C / 5% CO_2 / 95% relative humidity)
- 37°C water bath
- Ice-cold water bath: insulated bucket ¾ full of ice, plus cold water up to the level of the ice.
- Swinging bucket centrifuge capable of maintaining an internal temperature of 4°C
- 70-95% ethanol in spray bottle for disinfection of work surfaces and materials
- 15 mL conical tube
- Plastic bulb-top transfer pipette
- 5 & 10 mL serological pipettes
- Liquid waste container with sufficient capacity for the volume of waste produced (in this case ~25-30 mL per cryopreserved vial)

ATTAINING CONSISTENT RESULTS WITH CELL SYSTEMS

Cell Systems recommends the use of our complete cell growth and passage systems to optimize your laboratory research. These proprietary media were created to generate the healthiest cells for your assessment. Use of other media may affect results.

These media can be procured separately or as a bundle at www.cell-systems.com.

Preparation for Thawing and Plating Cells:

1. Chill 14mL supplemented growth medium in a 15mL conical tube in the ice water bath. To avoid possible contamination, make sure that water level never reaches the level of the tube's cap.
2. Warm the Attachment Factor™ and supplemented growth medium to 37°C in the warm water bath.
3. Spray down the outside of all tools/instruments (bottles, tubes, racks, etc.) with the ethanol solution before bringing them under the hood. Make sure all work surfaces are disinfected. Spray the outside of your gloves prior to work under the hood.
4. Prepare your flask(s):
    - Pipette Attachment Factor™ onto growth surface of flask ensuring full coverage (5mL per T-75 flask); wait ~5 seconds and then remove and discard.

- When preparing multiple flasks, you can use the same Attachment Factor™ sequentially in each flask.
- Add your desired volume of warm (37°C) supplemented growth medium to the T-75 flask. Aim for a final volume of 30 mL in the flask by adding 28 mL at this step.
- Place prepared flask(s) into 37°C incubator to maintain temperature.

5. Maintain the bottle of growth medium at 37°C for later use.

Procedure for Thawing and Plating Cells:

1. *Careful observation is required here.* Thaw the cryopreserved vial to 0°C in your 37°C water bath. Make certain that the water does not reach the cap of the vial.
- The goal is to maintain the temperature of the cells in the vial at <0°C until the moment they are placed into the 37°C medium in the culture flask.
- The optimal time to take the flask from the incubator to the hood is when the vial contains only a very small amount of frozen material left.

2. As soon as the vial has thawed, immediately disinfect the outside of the vial with the ethanol solution and move under the hood.

3. Using your bulb top transfer pipette, carefully remove the entire volume of cells from the vial and add to the ice-cold 14mL medium in the conical tube.
- For best results after transferring the cells, wash the inside of the vial with some of the medium from the conical tube in order to get as many cells out as possible.

4. Cap the conical tube and place into cooled swing bucket centrifuge. Centrifuge at 900 g and 4°C for 10 min.

5. Just before centrifugation is complete, move the prepared flask(s) from the incubator to under the hood.

6. When the centrifugation has finished, immediately remove the conical tube, disinfect with ethanol and move under the hood.

7. Carefully remove medium supernatant from the tube by aspirating or decanting (pouring), ensuring that you do not disturb the cell pellet at the bottom of the tube.

8. Pipette 200uL of CultureBoost™ onto the cell pellet and lightly flick with your finger in order to loosen the pellet and gently mix the CultureBoost™ with the cells. Allow the mixture to sit for no more than 10 seconds.
- This step is intended to expose the cells to a high concentration of growth factor and serum for a very short period and move them towards an active culture.

9. Re-suspend the cells in the appropriate amount of warm (37°C) growth medium using a serological pipette (e.g. 2mL). Gently draw the cell suspension up and down in order to mix and break up any cell aggregates.

10. Transfer the entire volume of cell suspension into your prepared flask(s) and place into 37°C incubator.