cell systems

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INSTRUCTIONS FOR FEEDING AND PASSAGING OF CELLS

Cell viability is affected by the efficiency with which you perform these processes. Cells are negatively affected by temperature fluctuations – severe damage to membrane and cytoskeletal components will result. Review your feeding or passaging process before initiation and move quickly and with purpose when working with these cells.

Materials Required for Feeding:

- Sterile lab gloves
- 37°C water bath
- 70-95% ethanol in spray bottle for disinfection of work surfaces and materials
- Cell Systems growth medium (appropriately supplemented with CultureBoost™ or RocketFuel™)
- Serological pipettes
- Liquid waste container with sufficient capacity for the volume of waste produced (for a T-75 flask, approx. 30 mL)

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 experiments. Use of other media may affect results.

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

Preparation for Feeding:

1. Warm the supplemented growth medium to 37°C in the water bath.
2. 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.

Procedure for Feeding:

1. Remove growth media from flask by pouring into waste receptacle or by aspiration.
2. Replace waste media with equal volume of pre-warmed supplemented growth media (10 mL for a T-25, 30 mL for a T-75 flask).
3. Return flask to incubator (37°C / 5% CO_2 / 95% relative humidity).

Materials Required for Passaging:

- Flask with cell culture at 80-90% confluence is optimal
- 7 ml per T-75 culture flask of each of Passage Reagent Group™ (PRG) 1, 2 & 3
- Suitable culture vessel, e.g. T-75 culture flask
- Attachment Factor™, 5 mL per 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 level of ice)

- Swing bucket centrifuge capable of maintaining an internal temperature of 4°C
- 70-95% ethanol in spray bottle for disinfection of work surfaces and materials
- 15mL conical tube
- Cell Systems growth medium (appropriately supplemented with CultureBoost™ or RocketFuel™)
- Serological pipettes
- Liquid waste container with sufficient capacity for the volume of waste produced (in this case ~50 mL per T-75 culture flask)

Preparation for Passaging:

1. Warm PRG1, PRG2, Attachment Factor™, and supplemented growth medium to 37°C in the warm water bath. Chill the PRG3 to ~0°C in the ice-cold water bath.
2. 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.
3. Prepare your flask(s):
   - Pipette Attachment Factor™ onto growth surface of flask ensuring full coverage (5 mL for 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 37°C growth medium into the T-75 flask. Aim for a final volume of 30 mL in the flask: if you plan to move 3mL of 9mL cell suspension (1:3 split) at Step 12, below, then add 27mL to the flask here.
   - Place prepared flask(s) into 37°C incubator to maintain temperature while cells are collected.
4. Maintain the bottle of growth medium at 37°C for later use.

Procedure for Passaging:

1. Remove growth medium from flask by pouring into waste receptacle or by aspiration.
2. Wash the culture by pipetting 7 mL of pre-warmed PRG1 into the flask and spreading across the entire growth surface. Then, remove PRG1 from flask by pouring into waste receptacle or by aspiration.
3. Pipette 7 mL of pre-warmed PRG2 into the flask and spread across the entire growth surface; immediately move to a microscope for observation.
4. As soon as ~90% of the cells in culture have rounded up, quickly return to the hood and add 7 mL of chilled PRG3 to the flask, ensuring that the entire growth surface is covered.
5. Pipette the cell suspension up and allow to flow down over the growth surface twice, to wash the surface and to collect any viable cells still adhering to the growth surface. If cells are sticking to the surface, sharply rapping the vessel should release them.
6. Transfer the entire cell suspension volume into a 15ml conical tube.
7. Centrifuge at 900 g and 4°C for 10 min.
8. Just before centrifugation is complete, move the prepared flask(s) from the incubator to under the hood.
9. When the centrifugation has finished, immediately remove the conical tube, disinfect with ethanol solution and move under the hood.
10. Carefully remove medium supernatant from the tube by aspiration or decanting (pouring), ensuring that you do not disturb the cell pellet at the bottom of the tube.
11. Re-suspend the cells in the desired volume of warm (37°C) growth medium using a serological pipette. Gently draw the cell suspension up and down in order to mix and break up any cell aggregates. For a typical 1:3 split, resuspend cells in 9mL and transfer 3mL of that in the following step.
12. Transfer the desired volume of cell suspension into your prepared flask(s) and place into incubator (37°C / 5% CO_2 / 95% relative humidity).