Jacket Lung Cell Model (CB-0301)
Passaging Protocol

A. Material

Product
500 mL RETM basal medium (CM-0001-A1)
15 mL RETM supplement (15 mL, thawed briefly in a 37°C water bath)(CM-0001-P1)
125 µL Cholera Toxin (100 µg/mL stock solution in cell culture grade water)
25 mL heat-inactivated Fetal Bovine Serum (FBS)(FBS-01-0100)
Tryple Express (Life Technologies #12604-021 recommended)
Quench solution: 20% FBS, 80% DMEM/F12 (EMD Millipore 227036 recommended)
Pen/strep (if desired)

B. Culture Conditions

1. Incubation in a multi-gas humidified 37°C incubator with 5% CO_2 in ambient air
2. Use of standard tissue culture-treated T-25 vent-cap flasks
3. A plating density of 300,000 cells/25 cm^2 should be used when passaging the cells. For initiating a culture from a frozen vial, see the Cell Growth Data (Table 1) for the recommended plating density.
4. Use of Renaissance Essential Tumor Medium (RETM) containing 5% heat-inactivated FBS
5. Recommended serum and Cholera Toxin
6. Dissociating cells in Tryple Express

C. RETM Complete Medium (5% FBS)

Combine the following to make RETM Complete Medium (5% FBS):
1. 500 mL RETM basal medium
2. 15 mL RETM supplement (15 mL, thawed briefly in a 37°C water bath)
3. 125 µL Cholera Toxin (100 µg/mL stock solution in cell culture grade water)
4. 25 mL heat-inactivated FBS
5. Pen/strep and/or other antibiotics (if desired)

Initiating a Culture from a Frozen Vial of Jacket Cells

I. Procedure

1. Complete RETM Complete Medium (5% FBS) by preparing it according to the instructions above (section C).
2. Prepare the reagent by transferring the growth medium from the refrigerator into a 37°C water bath until warmed. Thoroughly spray all components with 70% alcohol and wipe dry before placing in hood. In the hood, ensure a cryovial rack, a loosely capped 15 mL conical tube, and a loosely capped media bottle are aligned for convenient and sterile work flow.
3. Remove the frozen cell stock from the liquid nitrogen refrigerator by placing the frozen vial in a bucket of dry ice.
   Note: If dry ice is not present, wait until all reagent and hood preparations are compete before removing frozen cell sample. Frozen cell sample must remain completely frozen until ready for thawing.
4. Thaw the frozen cell suspension by removing the sample from the dry ice bucket and holding the frozen cell sample in the 37°C water bath until no less than 1/3 of cryovial contents are frozen.
   Note: Do not let water in bath contact the cap.
   Dry cryovial with paper towel. Spray cryovial with 70% alcohol, wipe dry and place in cryovial rack under the hood.
5. Immediately dilute the thawed cells in media by first uncapping the media bottle, 15 mL conical tube, and partially thawed cell sample in cryovial. Then, with a 10 mL sterile serological pipette, take up a volume of 10 mL media and immediately take up all liquid from frozen cell suspension cryovial.
6. Dispense 5 mL of serological pipette contents into the 15 mL conical tube.
7. With remaining serological pipette contents, rinse cryovial once and re-collect liquid. Dispense all serological pipette contents into the 15 mL conical tube. Tightly cap and invert to mix while carrying to centrifuge.

8. Centrifuge the conical tube at 500 x g for 5 minutes. Observe proper pellet formation.
   Note: Pellets should be opaque with a defined boundary and can have peaks but should be few with well-defined edges. If a pellet appears translucent and poorly defined, check centrifuge settings and centrifuge further.
9. Aspirate the supernatant, then invert the tube on a downward angle and aspirate the fluid draining away from the pellet.
10. Resuspend pellet in 10 mL RETM Complete Medium (5% FBS).
11. For plating the Jacket cells, consult the Cell Growth Data (Table 1) to find the recommended number of flasks to be plated. Label the appropriate number of new tissue culture-treated T-25 flask/flasks with information such as "Cell line; Growth Surface; Plating date; Passage number; Growth Media Name." Plating frozen cells adds one passage to the number on the vial.
12. Plate the 10 mL split appropriately among the recommended number of flasks.
    Note: If multiple flasks are plated, bring the media volume up to 10 mL per T-25 flask.
13. Immediately store newly cultured flask/flasks directly on the metal incubator shelf.

II. Notes

Expect a confluent flask based on the information shown in the Cell Growth Data (Table 1). Check degree of confluence each day and do not allow the flask to become more than 80% confluent. This prevents cluster formation on the edges that be difficult to dissociate.
Note: If the cells are less than 80% confluent, change media the following day and every 2–3 days thereafter (see section III). Media that appears yellow or yellow-orange must be changed. Media should never be allowed to become yellow.
When an 80% confluent flask is reached, cells are to be passaged per the procedure I-A.

III. 24 Hours after Plating Thawed Cells

For Jacket cells, collect the floating cells by centrifugation (500 x g for 5 minutes) prior to feeding. Perform as follows:
1. Collect the cell medium in 15 mL conical tube.
2. Add 10 mL RETM Complete Medium (5% FBS) to the flask and temporarily return to the incubator.
3. Centrifuge the conical tube at 500 x g for 5 minutes.
4. Return the conical tube and flask to the hood.
5. Aspirate the supernatant.
6. Use the medium in the flask to resuspend the pellet.
7. Add the medium and cells back to the flask and return to incubator.

Passaging Jacket Cultures in RETM Complete Medium (5% FBS)

Passaging is performed when culture flasks exhibit roughly 80% confluency. This protocol outlines the procedure for passaging Jacket cells in RETM Complete Medium (5% FBS) prepared according to the instructions in section C.

It is the responsibility of the Laboratory Manager and Safety Officer to ensure all laboratory personnel are properly trained in and follow this SOP. RETM Complete Medium (5% FBS) contains Cholera Toxin. Use 10% bleach to clean up any spills then dispose of materials as biohazardous waste. Lab coat and gloves are required.

I-A. Procedure

1. Prepare the reagent by placing Tryple and RETM Complete Medium (5% FBS) in a 37°C water bath until warmed. Keep Quench solution at 4°C until ready to use. Thoroughly spray all bottles with 70% alcohol and wipe dry before placing in hood.
2. To collect Jacket floating cells and dissociate the Jacket monolayer, use a 10 mL pipette to transfer the medium and floating cells in a culture flask to a 15 mL conical tube.
3. Add 2 mL Tryple to the flask and leave in room temperature.
4. Centrifuge the culture medium at 500 x g for 5 minutes. During the centrifugation, observe the flask under a microscope and incubate at 37°C and 5% CO_2 for 1–2 minutes
   Note: Do not over-incubate.
5. Check Jacket flask with microscope after incubation period. Ensure all cells have a bright, rounded appearance.
6. Knock flask on bench vigorously to release all cells from the surface. Check if cells are suspended by rapidly moving the flask across the microscope stage. Suspended cells will appear to keep moving once the flask has stopped.
   Note: Do not keep cells in contact with Tryple for over 5 minutes.
   If all cells appear to be suspended, then place flask in the hood for quenching.
7. Quench the Tryple by adding 8 mL of Quench solution to the flask, rinse the flask sides, mix by pipetting, and then add to a new 15 mL conical tube.
8. Aspirate the supernatant from the floating cell tube.
9. Resuspend the pellet in 1 mL Tryple and incubate at 37°C and 5% CO_2 for 1–2 minutes. Transfer dissociated floating cells in Tryple to the conical tube containing Quench and dissociated monolayer cells. Mix to combine.
10. Transfer ~300 µL to a microcentrifuge tube for counting. Cap the 15 mL conical tube and place in centrifuge at 500 x g for 5 minutes. Count the cells using a hemocytometer or counting method of choice.

11. To plate the tissue culture, label flask with information such as "cell line name; growth surface; plating date; passage number; # cells seeded; growth media name."
12. Calculate the volume needed to plate 300,000 cells per T-25 flask.
13. Mix cells in resuspended sample using a 5 mL pipette.
14. Add the cell suspension by measuring the calculated amount carefully with the appropriate size pipette.
15. Add RETM Complete Medium (5% FBS) to reach a 10 mL total flask volume.
16. Immediately store newly cultured flask directly on the metal incubator shelf.

II-A. Notes

Expect a confluent flask based on the information shown below (Table 1). Check degree of confluence each day and do not allow the flask to become more than 80% confluent. This prevents cluster formation on the edges that be difficult to dissociate.
Note: If the cells are less than 80% confluent change media the following day and every 2–3 days thereafter (see section III-A). Media that appears yellow or yellow-orange must be changed. Media should never be allowed to become yellow.
When an 80% confluent flask is reached, cells are to be passaged per the procedure I-A.

III-A. 24 Hours after Passaging

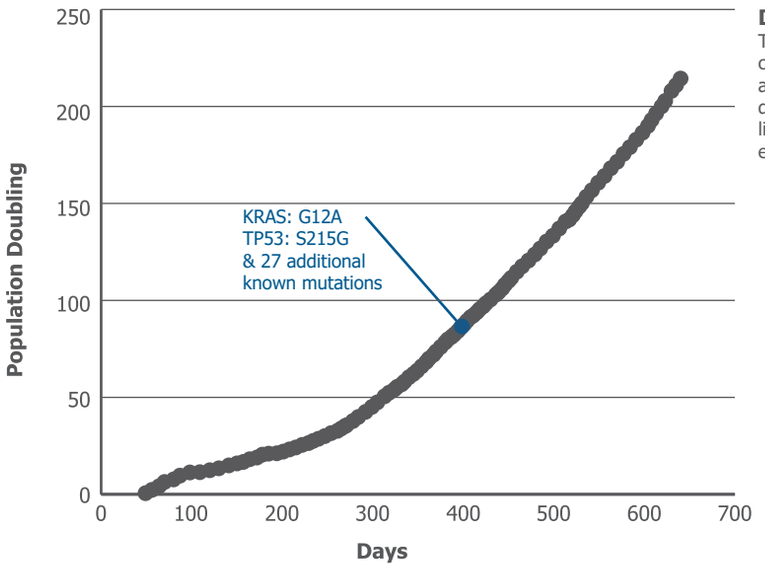
For Jacket cells, collect the floating cells by centrifugation (500 x g for 5 minutes) prior to feeding. Perform as follows:
1. Collect the cell medium in 15 mL conical tube.
2. Add 10 mL RETM Complete Medium (5% FBS) to the flask and temporarily return to the incubator.
3. Centrifuge the conical tube at 500 x g for 5 minutes.
4. Return the conical tube and flask to the hood.
5. Aspirate the supernatant.
6. Use the medium in the flask to resuspend the pellet.
7. Add the medium and cells back to the flask and return to incubator.

Table 1. Cell Growth Data

Product	Number Seeded	Number Harvested	Days Elapsed
1	1,000,000	1,560,000	1
2	300,000	940,000	3
3	300,000	1,000,000	3

Note: One vial was thawed and plated in a T-25 flask. This culture was passaged three times in RETM per the SOP. If followed, similar growth rates may be achieved.

Graph 1. Growth Curve



Doubling Time: 1.7 days
This growth curve demonstrates consistent growth and indicates a lack of cell crisis. It also demonstrates a continuous cell line accomplished without genetic engineering.