PASSAGING PROTOCOL
Ferry Colon Cell Model

CELL MODEL NAME: Ferry

Derived from a colon adenocarcinoma.

Product Category: Gorgione Colon Models

Catalog #: CB020-000001

Please refer to Certificate of Analysis for the lot in use.
This protocol was last modified on **January 13, 2017**

Record your **lot number** here:

FERRY CELL PASSAGING PROTOCOL OVERVIEW

Culture conditions will include:

- Incubation in a multigas humidified 37°C incubator with 5% CO_2 and 5% O_2
- Use of standard tissue culture-treated T-25 vent-cap flasks (such as Corning cat # 353109)
- A plating density of 120,000 cells/25 cm^2 should be used when passaging the cells. For initiating a culture from a frozen vial the Cell Growth Data section of the Certificate of Analysis for the current lot of cells should be consulted for the recommended plating density.
- Use of Renaissance Essential Tumor Medium (RETM) containing 7% heat inactivated, characterized FBS

  Recommended serum (Hyclone cat# SH30071.03), and Cholera Toxin (EMD Millipore cat # 227036-1MG). ***Please reference instructions below.*** *This prepared media will now be referenced as Complete RETM Culture Medium for the rest of this protocol.*
- Dissociating cells in Tryple Express (such as Life Technologies cat# 12604-021)

Recommended additional supplies

- Characterized FBS (Recommended serum: Hyclone cat # SH30071.03)
- Tryple Express (such as Life Technologies cat # 12604-021)
- Cholera Toxin (EMD Millipore cat # 227036-1MG)
- Quench Solution: 20% FBS (any FBS such as Atlas Biologicals cat # F-0500-A), 80% DMEM/F12 (such as Life Technologies cat # 11330-032)
- Pen/strep if desired

To make Complete RETM Culture Medium

Please combine the following:

- 500 mL RETM Basal Medium
- 15 mL RETM Supplement (15 mL, thawed briefly in a 37°C water bath)
- 125 uL Cholera Toxin (100 ug/mL stock solution in cell culture grade water)

  Recommended product: EMD Millipore cat # 227036-1MG
- 35 mL heat-inactivated fetal bovine serum

  Recommended product: Hyclone cat # SH3007103HI
- Pen-strep or other antibiotics as desired

1. INITIATING A CULTURE FROM A FROZEN VIAL OF FERRY CELLS

A. REAGENTS

i. Complete RETM Culture Medium (7% FBS) prepared according to the instructions above. Please reference for additional additives.

B. REAGENT PREPARATION

i. Place growth medium from refrigerator into 37°C water bath until warmed.
ii. Thoroughly spray bottle with 70% alcohol and wipe dry before placing in hood.

C. PREPARATION OF HOOD FOR EFFICIENT WORK-FLOW

i. In the hood, ensure a cryovial rack, a loosely capped 15mL conical tube, and a loosely capped media bottle are aligned for convenient and sterile work flow.

D. REMOVE FROZEN CELL STOCK FROM LIQUID NITROGEN REFRIGERATOR

i. Fill ice bucket with dry ice, and place frozen vial in the bucket.
    1. If dry ice is not present, wait until all reagent and hood preparations are compete before removing frozen cell sample.
    2. Frozen cell sample must remain completely frozen until ready for thawing.

E. THAW FROZEN CELL SUSPENSION IN CRYOVIAL

i. Remove frozen cell sample from dry ice bucket.
ii. Hold frozen cell sample in the 37°C water bath until no less than 1/3 of cryovial contents are frozen. Do not let water in bath contact the cap.
iii. Dry cryovial with paper towel.
iv. Spray cryovial with 70% alcohol, wipe dry and place in cryovial rack under the hood.
v. *Begin step **F. DILUTE THAWED CELLS IN MEDIA** immediately.*

F. DILUTE THAWED CELLS IN MEDIA

i. Uncap the following items:
    1. Media bottle
    2. 15mL conical tube
    3. Partially thawed cell sample in cryovial
ii. 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.
iii. Dispense 5mL of serological pipette contents into the 15 mL conical tube.
iv. With remaining serological pipette contents, rinse cryovial once and re-collect liquid.
v. Dispense all serological pipette contents into the 15 mL conical tube.
vi. Tightly cap 15 mL conical tube and invert to mix while carrying to centrifuge.

G. CENTRIFUGE FRESHLY THAWED FERRY CELL SUSPENSION

i. Centrifuge conical tube at 500 x g for 5 minutes.
ii. Observe proper pellet formation.
    1. Pellets should be opaque with a defined boundary.
    2. Pellets can have peaks but should be few with well-defined edges.
    3. If a pellet appears translucent and poorly defined, check centrifuge settings and centrifuge further.
iii. Aspirate the supernatant.
    1. Begin by aspirating supernatant and then inverting the tube on a downward angle and aspirating fluid draining away from the pellet.
iv. Re-suspend pellet in 10 mL Complete RETM (7% FBS).

H. PLATING FERRY CELLS

i. Consult the Cell Growth Data in the Certificate of Analysis for the current lot of cells 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."
    1. Plating frozen cells adds one passage to the number on the vial
ii. Plate the 10 ml split appropriately among the recommended number of flasks. If multiple flasks are plated bring the media volume up to 10 ml per T-25 flask.
iii. Immediately store newly cultured flask/flasks directly on the metal incubator shelf.

I. NOTES ON CULTURING CELLS

i. Expect a confluent flask based on the time line laid out in the lot specific Certificate of Analysis. Check degree of confluence each day, and don't allow the flask become more than 80% confluent to prevent cluster formation on the edges. These clusters may be difficult to dissociate.
ii. **If the cells are less than 80% confluent change media the following day referring to section J (CARE 24 HOURS AFTER PLATING THAWED CELLS)** and **every 2-3 days thereafter**. Media that appears yellow or yellow-orange must be changed. Media should never be allowed to become yellow.
iii. When an 80% confluent flask is reached cells are to be passaged per the protocol **PASSAGING FERRY CULTURES IN COMPLETE RENAISSANCE ESSENTIAL TUMOR MEDIUM**.

J. CARE 24 HOURS AFTER PLATING THAWED CELLS

For Ferry cells, collect the floating cells by centrifugation (500 x g for 5 minutes) prior to feeding. Perform as follows:

i. Collect the cell medium in 15 mL conical tube.
ii. Add 10 mL Complete RETM 7% FBS medium to the flask and temporarily return to the incubator.
iii. Centrifuge the conical tube at 500 x g for 5 minutes.
iv. Return the conical tube and flask to the hood.
v. Aspirate the supernatant.
vi. Use the medium in the flask to resuspend the pellet.

vii. Add the medium and cells back to the flask and return to incubator.

2. PASSAGING FERRY CULTURES IN COMPLETE RENAISSANCE ESSENTIAL TUMOR MEDIUM (RETM)

PURPOSE: Passaging is performed when culture flasks exhibit roughly 80 % confluency. This SOP outlines the procedure for passaging Ferry cells in Complete RETM Culture Medium (7% FBS) prepared according to the instructions above.

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

REAGENTS REQUIRED

- Complete RETM Culture Medium (7% FBS) prepared according to instructions on page 1 TO MAKE COMPLETE RETM CULTURE MEDIA
- Tryple Express: Life Technologies cat # 12604-021
- Quench Solution: 20% FBS (any FBS such as Atlas Biologicals cat # F-0500-A), 80% DMEM/F12 (such as Life Technologies cat # 11330-032)

PASSAGING PROCEDURE

A. REAGENT PREPARATION

i. Place Tryple and Complete RETM (7% FBS) in 37°C water bath until warmed.
ii. Keep Quench Solution at 4°C until ready to use.
iii. Thoroughly spray all bottles with 70% alcohol and wipe dry before placing in hood.

B. PREPARATION BEFORE WORKING IN THE HOOD

i. Spray with 70% alcohol and place the following items in the hood:
   1. 1.5mL Eppendorf tubes
   2. Micropipettes
   3. Micropipette tips
   4. Black fine alcohol proof pen
   5. Tryple Express
   6. Reagents from section A

C. COLLECT FERRY FLOATING CELLS AND DISSOCIATE FERRY MONOLAYER

i. Use a 10 ml pipette to transfer medium and floating cells in culture flask to a 15 mL conical tube.
ii. Add 2 mL Tryple to the flask and leave in room temperature.
iii. Centrifuge the culture medium at 500 x g for 5 minutes.
iv. During the centrifugation observe flask under microscope and incubate at 37°C/5% CO_2 for 1 to 2 minutes, do not over incubate.
v. Check Ferry flask with microscope after incubation period.
    1. Ensure all cells have a bright, rounded appearance.
vi. Knock flask on bench vigorously to release all cells from flask surface.
    1. 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. Check the edges of the flask.
    2. Do not keep cells in contact with Tryple for >5 minutes.
vii. If all cells appear to be suspended then place flask in the hood for quenching.
viii. Quench the Tryple by adding 8 mL of Quench solution to flask and rinse flask sides, mix by pipetting, and then add to a new 15 ml conical tube.
ix. Aspirate the supernatant from the floating cell tube.
x. Resuspend the pellet in 1 mL Tryple and incubate at 37°C/5% CO_2 for 1 to 2 minutes.
i. Transfer dissociated floating cells in Tryple to the conical tube containing Quench and dissociated monolayer cells. Mix to combine.
ii. Transfer ~300 ul to a micro centrifuge tube for counting.
    1. Cap the 15 mL conical tube and place in centrifuge at 500 x g for 5 minutes.
    2. Count the cells using a hemocytometer, or counting method of choice.

D. PLATE TISSUE CULTURE FLASKS WITH FERRY CELLS

i. Label flask with information such as “cell line name; growth surface; plating date; passage number; # cells seeded; growth media name.”
ii. Calculate the volume needed to plate 120,000 cells per T-25 flask.
iii. Mix cells in resuspended sample using a 5 mL pipette.
iv. Add the cell suspension by measuring the calculated amount carefully with the appropriate size pipette.
v. Add Complete RETM (7% FBS) to reach 10 mL total flask volume.
vi. Immediately store newly cultured flask directly on the metal incubator shelf.

E. NOTES ON CULTURING CELLS

i. Expect a confluent flask based on the time line laid out in the lot specific Certificate of Analysis. Check degree of confluence each day, and don’t allow the flask become more than 80% confluent to prevent cluster formation on the edges. These clusters may be difficult to dissociate.
ii. **If the cells are less than 80% confluent change media the following day referring to section F (INSTRUCTIONS FOR 24 HOURS AFTER PASSAGING)** and **every 2-3 days thereafter**. Media that appears yellow or yellow-orange must be changed. Media should never be allowed to become yellow.
iii. When an 80% confluent flask is reached cells are to be passaged per the protocol

PASSAGING FERRY CULTURES IN COMPLETE RENAISSANCE ESSENTIAL TUMOR MEDIUM.

F. INSTRUCTIONS FOR 24 HOURS AFTER PASSAGING

For Ferry cells, collect the floating cells by centrifugation (500 x g for 5 minutes) prior to feeding. Perform as follows:

i. Collect the cell medium in 15 mL conical tube.
ii. Add 10 mL Complete RETM medium to the flask and temporarily return to the incubator.
iii. Centrifuge the conical tube at 500 x g for 5 minutes.
iv. Return the conical tube and flask to the hood.
v. Aspirate the supernatant.
vi. Use the medium in the flask to resuspend the pellet.
vii. Add the medium and cells back to the flask, and return to incubator.