Cellaria

PASSAGING PROTOCOL
Wood Breast Cell Model

CELL MODEL NAME: Wood

Derived from an infiltrating ductal and lobular carcinoma of the breast.

Product Category: Cellini Breast Models

Catalog #: CB040-000001

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:

WOOD CELL PASSAGING PROTOCOL OVERVIEW

Culture conditions will include:

- Incubation in a multigas humidified 37°C incubator with 5% CO_2 in ambient air
- Use of standard tissue culture-treated T-25 vent-cap flasks (such as Corning cat # 353109)
- Using 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 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 5% 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)
- HBSS (1x) Hanks' Balanced Salt Solution (such as Gibco 14175)

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
- HBSS (1x) Hanks' Balanced Salt Solution (such as Gibco 14175)

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
- 25 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 WOOD CELLS

A. REAGENTS

i. Complete RETM Culture Medium (5% FBS).

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 15 mL 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. 15 mL conical tube
    3. Mostly-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 the thawed 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 WOOD 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 sharply 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 out of the pellet.
iv. Re-suspend pellet in 10 mL Complete RETM.

H. PLATING WOOD 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. ASSESS WOOD CELLS 24H AFTER PLATING

i. Examine Wood cells under the microscope and reference the growth curve in the Certificate of Analysis. Expect a confluent flask based on the time line laid out in the lot specific Certificate of Analysis. Check degree of confluence, and don't allow the flask become more than 80% confluent.
   1. If ready to split, move on to next section **PASSAGING WOOD CULTURES IN RENAISSANCE ESSENTIAL TUMOR MEDIUM**.
   2. If Not ready to split, move on to next step **J FEEDING WOOD CELLS**.

J. FEEDING WOOD CELLS

i. Aspirate the media.
ii. Add 5 ml HBSS(+) and rock the flask slowly to wash all sides of the flask.
iii. Aspirate HBSS(+).
iv. Add 10 mL fresh Complete RETM to the flask, and return to incubator.

K. NOTES ON CULTURING CELLS

i. Change media following the instructions in Section **J FEEDING WOOD CELLS** and repeat every 2-3 days thereafter. Media that appears yellow or yellow-orange must be changed. Media should never be allowed to become yellow.
ii. When a near-confluent flask is reached, passage the cells per the protocol **PASSAGING WOOD CULTURES IN RENAISSANCE ESSENTIAL TUMOR MEDIUM**.

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

PURPOSE: Passaging is performed when culture flasks exhibit roughly 80% confluency. This protocol outlines the procedure for passaging Wood cells in Complete RETM Culture Medium (5% 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 protocol. Complete RETM Culture Medium contains cholera toxin. Use 10% bleach to clean up any spills then dispose of materials as biohazardardous waste. Lab coat and gloves are required.

REAGENTS REQUIRED

- Complete RETM Culture Medium (5% 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)
- HBSS (1x) Hanks' Balanced Salt Solution (such as Gibco 14175)

PASSAGING PROCEDURE

A. REAGENT PREPARATION

i. Place Tryple Express 0.25% and Complete RETM 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. HBSS(-)
   7. Reagents from section A

C. RINSE MONOLAYER OF CULTURED FLASKS WITH HBSS(-)

i. Aspirate medium in culture flask.
ii. Add **5mL HBSS(-).**
iii. Rinse flask inside surfaces by rocking flask.

D. DISSOCIATE WOOD MONOLAYER

i. Aspirate HBSS (-).
ii. Add 2 mL Tryple Express to the flask.
iii. Incubate flask at 37°C /5% CO_2 for not more than 10 minutes checking after 5 minutes.
iv. While flask is incubating spray cold Quench Solution with alcohol and wipe dry before placing in the hood.
v. Check Wood 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 >10 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 15 ml conical tube.
ix. Transfer ~300 ul to a micro conical 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.

E. PLATE TISSUE CULTURE FLASKS WITH WOOD 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 300,000 cells per T-25 flask.
iii. Mix cells using a 5 mL pipette immediately before plating.
iv. Add the cell suspension by measuring the calculated amount carefully with the appropriate size pipette.
v. Add Complete RETM to reach 10 mL total flask volume.
vi. Immediately store newly cultured flask directly on the metal incubator shelf.

F. ASSESS WOOD CELLS 24H AFTER PLATING

i. Examine Wood cells under the microscope and reference the growth curve in the Certificate of Analysis. Expect a confluent flask based on the time line laid out in the lot specific Certificate of Analysis. Check degree of confluence, and don't allow the flask become more than 80% confluent.
    1. If ready to split, repeat protocol PASSAGING WOOD CULTURES IN RENAISSANCE ESSENTIAL TUMOR MEDIUM.
    2. If Not ready to split, move on to next step **G FEEDING WOOD CELLS**.

G. FEEDING WOOD CELLS

i. Aspirate the media.
ii. Add 5 ml HBSS(+) and rock the flask slowly to wash all sides of the flask.
iii. Aspirate HBSS(+).
iv. v. Add 10 mL fresh Complete RETM to the flask, and return to incubator.

H. NOTES ON CULTURING CELLS

i. Change media following the instructions in Section **G FEEDING WOOD CELLS** and repeat

every 2-3 days thereafter. Media that appears yellow or yellow-orange must be changed. Media should never be allowed to become yellow.

ii. When a near-confluent flask is reached, passage the cells per the protocol **PASSAGING WOOD CULTURES IN RENAISSANCE ESSENTIAL TUMOR MEDIUM**.