TECHNICAL MANUAL

GyneCult™ Fallopian Tube Organoid Medium

Table of Contents

1.0 Introduction

Fallopian tubes (FTs) are paired organs that connect each ovary to the uterus. They consist of a muscular outer layer enveloping an inner mucosa comprised of a single pseudostratified layer of epithelium. The luminal space encased by the mucosa serves as the site of fertilization and the path through which gametes or zygotes travel to and from the uterus. Therefore, the health and motility the cells contained therein rely on the secretions and ciliary motion of the two major FT epithelial cell types, secretory and ciliated cells, respectively. The FTs are also the site of many human maladies, including endometriosis, ectopic pregnancies, genetic disorders, infectious diseases, and cancer (Labidi-Galy et al., 2017; Lenz & Dillard, 2018; Pectasides et al., 2006). Among the diseases involving the FTs, high-grade serous ovarian carcinoma (HGSOC) is the most significant, both in its clinical impact and in research interest. Disease modeling of HGSOC is a major focus of FT-related research.

However, FT in vitro model systems—namely two-dimensional (2D) or air-liquid interface primary (ALI) cell cultures—are unable to fully recapitulate the FTs. The former lacks functional ciliated cells, supporting only the more stem-like and proliferative secretory cells, and the latter does not support self-renewal, as ALI cultures cannot be biobanked or passaged (Fotheringham et al., 2011; Karst & Drapkin, 2012). HGSOC modeling, on the other hand, has focused on patient-derived xenografts in vivo and the establishment of 2D cell lines in vitro. While xenografts remain a reliable model for HGSOC, their establishment and maintenance are cost-prohibitive, especially for studies requiring adequate disease sampling and replication of experimental conditions. Traditional in vitro approaches have led to the establishment of many cell lines currently used for HGSOC research; however, there are legitimate concerns surrounding their modeling efficacy and provenance, as it has been demonstrated that many heavily cited HGSOC cell lines are, in fact, genomically distinct from true HGSOC (Bell et al., 2011; Domcke et al., 2013).

GyneCult™ Fallopian Tube Organoid Medium (FTOM) is a four-component, serum- and phenol red-free medium for the long-term expansion of primary human FT cells in matrix-embedded organoid cultures. It is fully compatible with fresh or cryopreserved dissociated human FT cells, with balanced representation of the two major FT lineages. It is also compatible with the long-term culture of at least ~33% of fresh or cryopreserved dissociated HGSOC cells.

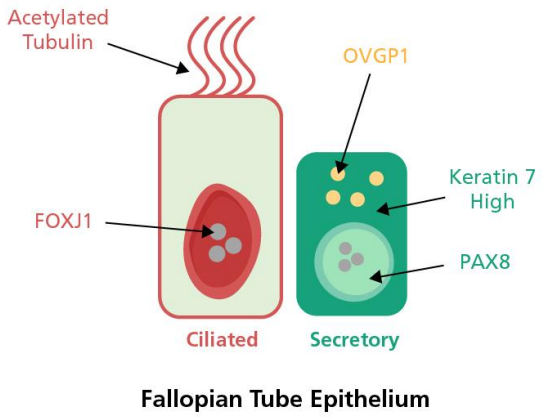

Figure 1. Marker Expression of Fallopian Tube Epithelial Cells

Overview of marker expression in ciliated and secretory FT epithelial cells. Ciliated FT cells express acetylated alpha tubulin (AcTub) and forkhead box protein J1 (FOXJ1). Secretory FT cells express paired box gene 8 (PAX8), oviductal glyoprotein 1 (OVGP1), and exhibit high expression of K7.

2.0 Materials, Reagents, and Equipment Required

2.1 GyneCult™ Fallopian Tube Organoid Media

The components listed below are sold as a complete kit and are not available for individual sale. For component storage and stability information, refer to the Product Information Sheet (PIS), available at www.stemcell.com, or contact us to request a copy.

PRODUCT NAME	CATALOG #	COMPONENT NAME	COMPONENT #	QUANTITY
GyneCult™ Fallopian Tube Organoid Medium	100-1245	GyneCult™ Fallopian Tube Organoid Basal Medium	100-1244	90 mL
		GyneCult™ Fallopian Tube Organoid Medium 5000X Supplement	100-1243	1.5 mL
		GyneCult™ Fallopian Tube Organoid Medium 100X Supplement	100-1246	1 mL
		GyneCult™ Fallopian Tube Organoid Medium 10X Supplement	100-1247	10 mL

2.2 Additional Required Materials and Reagents

PRODUCT	CATALOG #
24-well non-treated plates	e.g. 38042
100 µm Reversible Strainer, Large	27270
125 mL or 250 mL dissociation flasks OR Conical tubes, 15 mL or 50 mL	e.g. Bellco Glass 1992-00125 (125 mL) or 27300 (250 mL) OR e.g. 38009 or 38010
Ammonium Chloride Solution	07800
Collagenase Type II OR Collagenase from *Clostridium histolyticum*	07418 OR Sigma C9407
Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix, Phenol Red-free, LDEV-free, 10 mL	Corning 356231
CryoStor® CS10	07930
Dispase (5 U/mL)	07913
DMEM/F-12 with 15 mM HEPES	36254
DNase I Solution (1 mg/mL)	07900
Fetal bovine serum	e.g. 100-0180
Glass Petri dish	e.g. VWR 75845-544
HBSS with 10 mM HEPES, Without Phenol Red	37150
Trypan Blue	07050
Trypsin-EDTA, 0.25% and 0.05%	07901 and 07910
Y-27632 (Dihydrochloride)	e.g. 72302
Optional: Antibiotics	--

For a complete list of products available from STEMCELL Technologies Inc., visit www.stemcell.com.

2.3 Equipment

- Biosafety cabinet certified for Level II handling of biological materials
- Incubator with humidity and gas control to maintain 37°C and 95% humidity in an atmosphere of 5% CO_2 in air
- Hypoxic incubator with humidity and gas control to maintain 37°C and 95% humidity in an atmosphere of 5% O_2 and 5% CO_2
- Low-speed centrifuge with a swinging bucket rotor
- Orbital shaker (e.g. Celltron INFORS HT I69222)
- Hemocytometer (e.g. Catalog #100-1181) or Nucleocounter®
- Multi-gas or tri-gas incubator
- Pipettor with appropriate tips (e.g. Catalog #38059)
- Pipette-Aid with appropriate serological pipettes (e.g. Catalog #38002)
- Inverted microscope
- Freezing containers
- -20°C freezer
- -150°C freezer or liquid nitrogen (LN2) vapor tank
- -80°C freezer
- Refrigerator (2 - 8°C)
- Sterile filtration unit

3.0 Preparation of Media

Prepare the following media as directed for use in organoid seeding and maintenance (section 6.0).

3.1 GyneCult™ Fallopian Tube Organoid Medium (FTOM)

Use sterile technique to prepare complete GyneCult™ FTOM (GyneCult™ Fallopian Tube Organoid Basal Medium + GyneCult™ Fallopian Tube Organoid Medium 10X Supplement + GyneCult™ Fallopian Tube Organoid Medium 100X Supplement + GyneCult™ Fallopian Tube Organoid Medium 5000X Supplement). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw GyneCult™ Fallopian Tube Organoid Medium 10X Supplement at room temperature (15 - 25°C) for 1 hour. Mix thoroughly, then store on ice.
2. Aliquot 89 mL of GyneCult™ Fallopian Tube Organoid Basal Medium into a sterile container.

   Note: If antibiotics are required, reduce basal medium volume to accommodate antibiotic volume.
3. Add 10 mL of GyneCult™ Fallopian Tube Organoid Medium 10X Supplement.

   Note: If not used immediately, aliquot supplement and store at -20°C. After thawing aliquots, use immediately; do not re-freeze. Do not exceed the expiry date as indicated on the label. Alternatively, store supplement at 2 - 8°C for up to 1 week.
4. Add 1 mL of GyneCult™ Fallopian Tube Organoid Medium 100X Supplement.

   Note: If not used immediately, aliquot supplement and store at -20°C. After thawing aliquots, use immediately; do not re-freeze. Do not exceed the expiry date as indicated on the label. Alternatively, store supplement at 2 - 8°C for up to 1 week.
5. Add 20 µL of GyneCult™ Fallopian Tube Organoid Medium 5000X Supplement. Mix thoroughly without generating bubbles.
6. Optional: Add antibiotic(s)/antimycotic(s) as desired.

   Note: If not used immediately, store complete medium at 2 - 8°C for up to 6 weeks. Do not exceed the expiry date of the individual components.

3.2 GyneCult™ FTOM for High Grade Serous Ovarian Carcinoma (HGSOC) (FTO-HM)

Use sterile technique to prepare complete GyneCult™ FTO-HM (GyneCult™ Fallopian Tube Organoid Basal Medium + GyneCult™ Fallopian Tube Organoid Medium 10X Supplement ± GyneCult™ Fallopian Tube Organoid Medium 5000X Supplement). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw GyneCult™ Fallopian Tube Organoid Medium 10X Supplement at room temperature (15 - 25°C) for 1 hour. Mix thoroughly, then store on ice.
2. Aliquot 90 mL of GyneCult™ Fallopian Tube Organoid Basal Medium into a sterile container.

   Note: If antibiotics are required, reduce basal medium volume to accommodate antibiotic volume.
3. Add 10 mL of GyneCult™ Fallopian Tube Organoid Medium 10X Supplement.

   Note: If not used immediately, aliquot and store supplement at -20°C. Do not exceed the expiry date as indicated on the label. After thawing aliquots, use immediately; do not re-freeze. Alternatively, store supplement at 2 - 8°C for up to 1 week.
4. Optional: Add 20 µL of GyneCult™ Fallopian Tube Organoid Medium 5000X Supplement. Mix thoroughly without generating bubbles.

   Note: The role of the 5000X supplement in the HGSOC culture may be donor-dependent.

5. Optional: Add antibiotic(s)/antimycotic(s) as desired.

   Note: If not used immediately, store complete medium at 2 - 8 °C for up to 6 weeks. Do not exceed the expiry date of the individual components.

4.0 Protocol Diagram

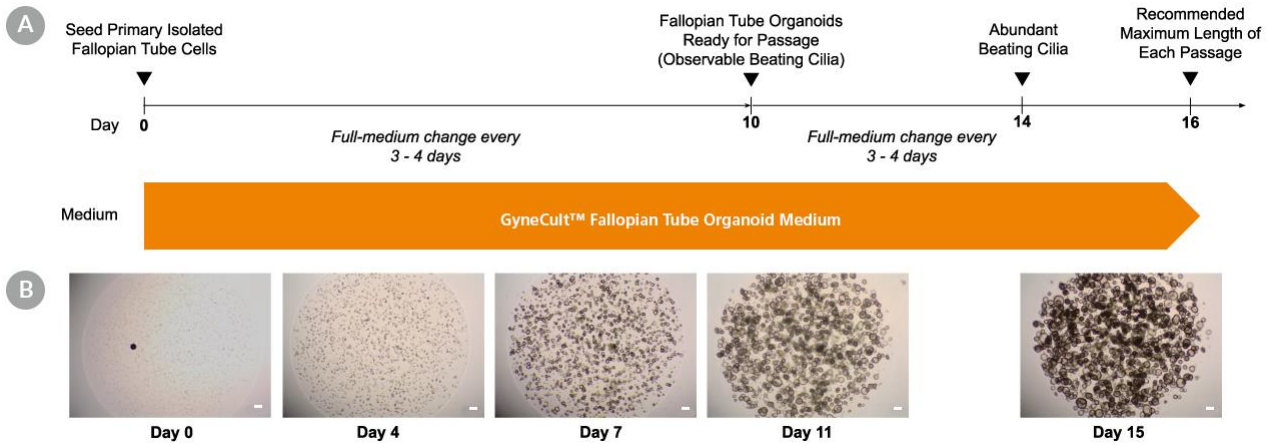

Figure 2. GyneCult™ FTOM Workflow Diagram and Representative Images of Expected Dome Culture Morphology. Scale bar = 200 µm.

5.0 Dissociation of Human Fallopian Tube and HGSOC Tumor Tissue

The following is a protocol to dissociate human FT or HGSOC surgical samples. This protocol is optimized for freshly isolated samples or samples that have been appropriately stored in a medium or buffer (e.g. DMEM/F-12 with 15 mM HEPES [DMEM/F-12] or HBSS with 10 mM HEPES, Without Phenol Red [HBSS]), ideally with 2 - 5% fetal bovine serum (FBS), for no more than 48 hours at 2 - 8°C.

5.1 Human Fallopian Tube and HGSOC Tissue Dissociation

1. Prepare the enzyme dissociation mixture as follows:

    a. First, prepare the 10X enzyme dissociation stock solution by dissolving Collagenase Type II at 50 mg/mL (6250 U/mL) into DMEM/F12 or HBSS.
    Note: Alternatively, Collagenase from Clostridium histolyticum can be used at 10 mg/mL to prepare the 10X enzyme dissociation stock solution.

    b. Incubate the stock solution at room temperature (15 - 25°C), with or without rotation or agitation, until the powder is dissolved (~15 - 30 minutes). Warm in a 37°C water bath if necessary.

    Note: If not used immediately, aliquot and store 10X enzyme dissociation stock solution at -20°C for up to 12 months.

    c. Dilute the 10X stock solution 1 in 10 in DMEM/F-12. The volume of 1X enzyme dissociation mixture required will depend on the volume of the sample to be dissociated (Table 1).
2. To prepare 500 mL of Wash Buffer, add 10 mL of FBS to 490 mL of HBSS. Mix thoroughly.

    Note: If not used immediately, store the Wash Buffer at 2 - 8ºC for 12 months.
3. Using sterile forceps, transfer the tissue from the transport medium into a pre-weighed 50 mL conical tube with sufficient volume of cold DMEM/F-12 to cover the tissue. If the tissue cannot be transferred using forceps, let gravity settle the tissue before aspirating the excess medium, then pour the tissue into the centrifuge tube. Determine the weight of the tissue by weighing the tube with the tissue and subtracting the initial weight of the tube.
4. Using forceps, transfer the tissue to a sterile glass Petri dish or decant the tissue with DMEM/F-12 and remove the medium from the Petri dish afterwards. Keep a small amount of medium in the Petri dish to prevent the tissue from drying out.
5. While wearing cut-resistant gloves, use scalpels to mince the tissue into ~ 1 mm pieces. Do not mince for longer than 10 minutes to prevent the tissue from warming up.
6. Using the scalpels or a sterile spatula, transfer the minced tissue to an appropriate dissociation vessel and add the enzyme dissociation mixture as directed in Table 1. Use additional vessels if necessary.
7. Rinse the Petri dish using a small volume of 1X enzyme dissociation mixture and combine with transferred tissue to minimize tissue loss, as necessary.

Table 1. Recommended volumes and vessels for dissociation

TISSUE SAMPLE VOLUME	ENZYME DISSOCIATION MIXTURE VOLUME	DISSOCIATION VESSEL
< 5 mL	10 mL	125 mL baffled dissociation flask or 15 mL conical tube*
5 - 15 mL	25 mL	125 mL baffled dissociation flask or 50 mL conical tube*
15 - 30 mL	~40 mL	125 mL baffled dissociation flask or 50 mL conical tube*

*Use of 125 mL baffled dissociation task is preferred.

8. Before incubating, seal the dissociation vessel by covering the opening of the flask with sterile aluminum foil or sealing with Parafilm®.
9. Incubate the dissociation vessel at 37°C for 1 hour on an orbital shaker set to 80 - 100 RPM in a CO_2 incubator. Triturate the enzyme dissociation mixture with a 5 or 10 mL pipette every 15 minutes. Cover and seal the vessel again with aluminum foil or Parafilm® after every trituration. Stop the dissociation when most or all of the fragments of tissue are broken down.
10. After dissociation, prepare the sample for centrifugation as follows:
    - Dissociation flask: Transfer the dissociated tissue sample to 50 mL conical tube(s). Wash the dissociation flask with an equal volume of Wash Buffer and add the wash to the same conical tube(s).
    - 50 mL conical tube: Top up to 50 mL total volume with Wash Buffer. Proceed to step 11.
    - 15 mL conical tube: Transfer the dissociated tissue sample to 50 mL conical tube(s). Wash the 15 mL conical tube with an equal volume of Wash Buffer and add the wash to the same 50 mL conical tube(s).
11. Centrifuge the tubes at 430 x *g* for 5 minutes at 2 - 8°C. Remove and discard the supernatant.
12. Add an additional volume (e.g. 10 - 25 mL) of Wash Buffer to each conical tube and resuspend the pellet. Do not vortex.
13. Centrifuge tubes again at 430 x *g* for 5 minutes at 2 - 8°C and discard the supernatant. This wash will remove any traces of digestive enzymes from the cells. Multiple tubes can be pooled into one tube at this point.
14. Resuspend pelleted cells in 1 mL of Wash Buffer and add 4 mL of Ammonium Chloride Solution (ACS). For large pellets, up to 3 mL of Wash Buffer can be used for resuspension, while increasing the added ACS volume to maintain a 4:1 ratio of ACS to Wash Buffer.

    Note: If resuspending in > 1 mL of Wash Buffer, use a 50 mL conical tube.
15. Incubate at room temperature (15 - 25°C) for 3 - 5 minutes, and then top up with 2 volumes of HBSS relative to the total volume of sample + Wash Buffer + ACS from step 14.
16. Centrifuge at 430 x *g* for 5 minutes at 2 - 8°C. Remove and discard the supernatant.
17. Proceed to section 5.2 to further dissociate samples into single cells, which is recommended (especially for FT samples).

    OR

    The HGSOC and FT cell clusters/clumps can be counted and seeded directly into cultures (proceed to section 5.3).

    Note: Alternatively, the HGSOC and FT cell clusters/clumps can be cryopreserved. For more information, proceed to section *5.4.*

5.2 Single-Cell Dissociation

1. Resuspend the pellet in 1 mL of 0.25% Trypsin and incubate in a 37ºC water bath for 3 - 5 minutes. Quench the reaction by adding 9 volumes of Wash Buffer to the tube.

   Note: For larger pellets, resuspend in up to 2 mL of 0.25% Trypsin.
2. Centrifuge at 430 x *g* for 5 minutes at 2 - 8°C and remove supernatant.
3. Resuspend the pellet in 1 mL of Dispase (use up to 2 mL for larger pellets). Add 100 µL DNAse I Solution (1 mg/mL) per mL of Dispase and incubate in a 37ºC water bath for 3 - 5 minutes. Quench the reaction by adding 9 volumes of Wash Buffer to the tube.

   Note: For larger pellets, resuspend in up to 2 mL of Dispase.
4. Optional: Strain the sample through a 100 µm cell strainer into a 50 mL conical tube. Wash the remaining cells in the strainer with 10 mL of Wash Buffer.
5. Centrifuge at 430 x *g* for 5 minutes at 2 - 8°C and remove supernatant.
6. Resuspend the pellet in 1 mL of Wash Buffer to obtain a single-cell suspension. Count live cells using Trypan Blue and a hemocytometer or Nucleocounter®.

   Note: For resuspending larger pellets, increase the volume of Wash Buffer as needed.
7. Add 10 mL of Wash Buffer to the cell suspension. Centrifuge at 430 x *g* for 5 minutes at 2 - 8°C and remove supernatant.

   Note: Alternatively, the single-cell suspension can now be cryopreserved. To cryopreserve cells, centrifuge and resuspend cells in CryoStor® CS10 at 0.5 - 2 x 10^6 cells/mL. Use a minimum of 200 µL of CryoStor® CS10 per vial for aliquots with fewer than 1 x 10^5 cells.
8. Resuspend the pellet in 1 mL of Wash Buffer to obtain a single-cell suspension. To initiate organoid cultures, proceed to section 6.2.

5.3 Cell Clump Culture Preparation

1. Add 10 mL of Wash Buffer into the tube(s) and resuspend the pellet(s).
2. Optional: Strain the sample through a 100 µm cell strainer into a 50 mL conical tube. Wash the remaining cells in the strainer with 10 mL of Wash Buffer.
3. Centrifuge at 430 x *g* for 5 minutes at 2 - 8°C.
4. Discard the supernatant and resuspend cells in 1 mL of Wash Buffer. Increase volume of Wash Buffer as needed for larger pellets.
5. Perform a live cell count using Trypan Blue and a hemocytometer or Nucleocounter®. To initiate organoid cultures, proceed to section 6.2.

5.4 Cryopreservation

1. Add 10 mL of Wash Buffer into the tube(s) and resuspend the pellet(s).
2. Optional: Strain the sample through a 100 µm cell strainer into a 50 mL conical tube. Wash the remaining cells in the strainer with 10 mL of Wash Buffer.
3. Centrifuge at 430 x *g* for 5 minutes at 2 - 8°C.
4. Discard the supernatant and resuspend cells in 1 mL of Wash Buffer. Perform a live cell count using Trypan Blue and a hemocytometer or Nucleocounter®.

5. Centrifuge tubes at 430 x *g* for 5 minutes at 2 - 8°C and discard the supernatant.
6. Resuspend cells in CryoStor® CS10 at 0.5 - 2 x 10^6 cells/mL. Use a minimum of 200 µL of CryoStor® CS10 per vial for aliquots with fewer than 1 x 10^5 cells. Use additional vials as needed.

6.0 Organoid Culture Protocol

6.1 Thawing Cryopreserved Cells

1. Prepare a 0.5 - 1 mL aliquot of cold complete GyneCult™ FTOM or FTO-HM for FT or HGSOC cells, respectively, per vial to be thawed. Keep at 2 - 8°C or on ice until use (step 7).

   Note: Wash Buffer can be used instead of the complete media.
2. Transfer all vials of cryopreserved cells (0.5 - 1 mL) for thawing from ultra-low temperature storage (e.g. liquid nitrogen) onto dry ice.
3. Process one tube at a time as follows:
   a. Take one vial off the dry ice and transfer it to a 37°C water bath. Swirl until only a very small amount of ice remains.
   b. Carefully pipette the thawed cells into a 15 mL conical tube.
   c. Add 1 mL of cold Wash Buffer into the empty vial to wash, pipetting up and down 3 times.
   d. Add the wash, dropwise, to the cells in the conical tube, swirling to ensure mixing with each drop.
   e. Continue to add additional cold Wash Buffer, dropwise, into the conical tube until cell suspension is 2.5 - 3X the original volume (e.g. for 1 mL of thawed cells, add Wash Buffer dropwise for a total volume of 2.5 - 3 mL).
   f. Slowly bring the volume up to 5 - 10X the original volume using cold Wash Buffer.
   g. Store on ice.
4. Centrifuge the tube(s) at 430 x *g* for 5 minutes at 2 - 8°C.
5. Aspirate the supernatant and gently resuspend each pellet in 1 mL of cold Wash Buffer using a 1 mL micropipette. Add an additional 4 mL of cold Wash Buffer to each tube and mix well.
6. Centrifuge the tubes at 430 x *g* for 5 minutes at 2 - 8°C.
7. Aspirate supernatant and gently resuspend each pellet in 0.5 - 1 mL of cold complete GyneCult™ FTOM or FTO-HM for FT or HGSOC cells, respectively (prepared in step 1).

   Note: Wash Buffer can be used instead of the complete media.
8. Aliquot the desired number of cells into sterile microcentrifuge tubes, up to 1 x 10^5 FT cells or 5 x 10^5 HGSOC cells per tube (i.e. to seed 4000 FT cells and 20,000 HGSOC cells per Matrigel® dome).

   Note: If cell numbers exceed stated numbers, use additional sterile microcentrifuge tubes at the same ratio of maximum cells per tube.
9. Store on ice. Proceed to section 6.2 for culture initiation.

6.2 Initiation of Human Fallopian Tube or HGSOC Organoid Cultures

The following protocol is for plating Matrigel® domes containing 4000 FT cells or 20,000 HGSOC cells per dome in a 24-well non-tissue culture-treated plate. If preparing a different number of Matrigel® domes, adjust volumes accordingly.

Example: To plate 20 domes, prepare enough cell/Matrigel® suspension for 22 - 24 domes to account for pipetting loss.

1. For every 4000 FT cells or 20,000 HGSOC cells to be plated, thaw 20 µL of Matrigel® on ice.

2. Centrifuge the microcentrifuge tube(s) containing freshly isolated (prepared in section 5.0) or thawed human FT or HGSOC cells (prepared in section 6.1) at 430 x *g* for 5 minutes at 2 - 8°C.
3. Aspirate the supernatant and add 20 µL of Matrigel® for every 4000 FT cells or 20,000 HGSOC cells in the tube(s). Pipette up and down 20 - 30 times, slowly but forcefully. Avoid generating bubbles. Store tubes on ice.
4. Using a 24-well non-tissue culture-treated plate, process one tube/plate at a time as follows:
    a. Working quickly and minimizing contact between fingertips and the tube, take the tube of Matrigel®-cell suspension off the ice. Using a 1 mL pipettor, pipette up and down 8 - 10 times quickly but gently to create a uniform suspension. Avoiding generating bubbles in the suspension by stopping at the first stop of the pipette.
    b. Using a 20 µL pipettor, transfer 20 µL of the Matrigel®-cell suspension (stopping at the first stop of the pipettor to avoid bubbles) to the center of the well to plate 1 dome/well, or off-center, such that each dome is equidistant from the center of the well to plate 3 domes/well.
    c. Repeat step b in the next well of the plate until the entire volume of Matrigel®-cell suspension is plated.
    d. Place the lid on the culture plate. Carefully transfer the plate to the incubator without disturbing the domes.
    e. Incubate the plate at 37°C for 15 - 20 minutes.

    Note: If preparing more than one microcentrifuge tube of thawed cells, step 4a can be started for the next tube and plated during this incubation period.
    f. Prepare a sufficient volume of complete GyneCult™ FTOM or FTO-HM per plated well for FT or HGSOC cells, respectively. Add Y-27632 (Dihydrochloride) to a final concentration of 10 µM. Warm to room temperature (15 - 25°C).
    g. Gently add 500 µL of room temperature complete medium + 10 µM Y-27632 (Dihydrochloride) to each well containing a dome. Gently pipette the medium against the wall of the well to avoid disturbing the dome(s).
    h. Carefully transfer plate to a 37°C and 5% CO_2 incubator. Do not disturb the plate for 3 - 4 days.

    Note: Incubate HGSOC cultures in a 5% O_2 and 5% CO_2 incubator.

6.3 Maintenance of Human Fallopian Tube or HGSOC Organoid Cultures

6. On day 3 or 4, perform a full-medium change by carefully aspirating all of the medium from each well, then adding 500 µL of fresh, room temperature (15 - 25°C) medium:
    - For FT organoid cultures, use GyneCult™ FTOM without Y27632
    - For HGSOC organoid cultures, use GyneCult™ FTO-HM + 10 µM Y-27632

    Note: Y-27632 can be kept in the medium for the duration of the FT culture without impacting performance.
7. Perform a full-medium change every 3 - 4 days.
8. Proceed to section 6.4 to passage organoid cultures as follows:
    - Passage FT organoid cultures between day 10 and day 14 after seeding.

      Note: Cultures should be passaged or harvested by day 16.
    - For HGSOC organoid cultures, passage when most growing organoids are greater than 100 µm in diameter, or by day 30.

6.4 Passaging Human Fallopian Tube or HGSOC Organoids

1. Warm 1 mL of 0.25% Trypsin to 37ºC per well to be passaged.

   Note: Use 0.05% Trypsin to passage HGSOC organoid cultures.

2. Without touching the dome(s), aspirate the culture medium completely for each well to be passaged.
3. Add 1 mL/well of 0.25% Trypsin Solution (i.e. per up to 60 µL Matrigel®-cell suspension).

   Note: Use 0.05% Trypsin to passage HGSOC organoid cultures.

4. Pipette up and down 10 times, forcefully and quickly, to shear large Matrigel® aggregates. Avoid generating bubbles by stopping at the first stop of the pipette.
5. Incubate the plate as follows:
   - For FT organoid cultures, incubate at 37°C for 10 minutes
   - For HGSOC organoid cultures, incubate at room temperature for 5 minutes
6. Aliquot 1 mL of cold Wash Buffer into a 15 mL conical tube for each well. Replicate wells can be pooled, while maintaining the same volume of Wash Buffer per well. For HGSOC organoids, proceed to step 8.
7. For FT organoids only: Using a 1 mL pipettor, triturate each well again 10 times. Incubate at 37°C for 10 minutes.
8. Pipette up and down 10 times. Add the dissociated cells to a conical tube containing 1 mL of cold Wash Buffer. Triturate 5 times to mix thoroughly. Replicate wells can be pooled while maintaining the same volume of Wash Buffer per well.
9. Wash the well with 1 mL of cold Wash Buffer by pipetting up and down 3 - 5 times. Transfer the wash to the conical tube containing the dissociated cells from that well. Pipette up and down again 3 - 5 times to mix. Replicate wells can be pooled if desired.
10. Centrifuge the tube(s) at 430 x *g* for 5 minutes at 2 - 8°C.
11. Aspirate the supernatant and resuspend the cells in a minimum volume of 250 - 500 µL of complete GyneCult™ FTOM or FTO-HM for FT or HGSOC cells, respectively. Mix by pipetting 5 - 8 times.

    Note: Wash Buffer can also be used to resuspend cells.

12. Perform a live cell count and aliquot desired number of cells into sterile microcentrifuge tubes, up to 1 x 10^5 FT cells or 5 x 10^5 HGSOC cells per tube (i.e. to seed 4000 FT cells and 20,000 HGSOC cells per Matrigel® dome).

    Note: If necessary, use additional sterile microcentrifuge tubes at the same ratio of maximum cells per tube.

    Note: Early cultures may display limited growth capacity. If there are < 4000 FT cells or < 20,000 HGSOC cells after counting, seed all available cells into 1 x 20 µL dome.

13. To seed into the next passage, refer to section 6.2.

7.0 Fallopian Tube or HGSOC Organoid Culture Evaluation

The following section details a protocol used to evaluate the organoids and cells grown with GyneCult™ FTOM or FTO-HM.

7.1 Organoid-Forming Efficiency Assay

Organoid-forming efficiency (OFE) is a measure of a culture's stemness, i.e. the percentage of cells in culture that successfully form organoids. In a typical healthy expansion organoid culture, an OFE of 5 - 20% is to be expected (tested across 5 independent donors). OFE is also a measure of self-renewal and organogenic capacity of the seeded cells that can serve as a readout for applications, such as drug screening and toxicity assays.

Refer to the following protocol to assess OFE:

1. Instead of seeding 1 - 3 x 20-µL domes/well (section 6.2 step 4b), seed 1 - 3 x 18-µL domes into each well, followed by up to 3 x 2-µL domes into the same well (i.e. 1 small dome per large dome). Alternatively, up to 3 small domes can be seeded for any number of large domes such that the total volume of suspension seeded is 20 µL, 40 µL, or 60 µL for wells containing 1, 2, or 3 large domes, respectively. For example, 3 x 2-µL small domes can be co-seeded with a 1 x 14-µL large dome in the same well (20 µL or 4000 cells total).

   Note: The small domes can be combined with the large dome during passaging and used in expansion after they are first quantified (on or after day 10) with the following steps before passage (section 6.4).
2. On an inverted microscope (4X objective), locate a seeded 2-µL dome.
3. Adjust focus so that all organoids are in focus or visible for accurate counting.
4. Count the number of organoids in the dome, or capture an image of the dome for counting at a later time.
5. Repeat steps 2 - 4 for each 2-µL dome seeded.
6. Calculate the OFE:

$$\textit{OFE (\%)} = \frac{\textit{Number of Organoids per Dome}}{\textit{Number of Cells Seeded per Dome}} \times 100$$

8.0 References

Bell D et al. (2011) Integrated genomic analyses of ovarian carcinoma. Nature 474(7353): 609–15.

Domcke S et al. (2013) Evaluating cell lines as tumour models by comparison of genomic profiles. Nat Commun 4: 1–10.

Fotheringham S et al. (2011) Ex vivo culture of primary human fallopian tube epithelial cells. J Vis Exp (51): 1–5.

Karst AM & Drapkin R. (2012) Primary culture and immortalization of human fallopian tube secretory epithelial cells. Nat Protoc 7(9): 1755–64.

Labidi-Galy SI et al. (2017) High grade serous ovarian carcinomas originate in the fallopian tube. Nat Commun 8(1): 1–10.

Lenz JD & Dillard JP. (2018) Pathogenesis of Neisseria gonorrhoeae and the Host Defense in Ascending Infections of Human Fallopian Tube. Front Immunol 9(NOV).

Pectasides D, Pectasides E, & Economopoulos T. (2006) Fallopian tube carcinoma: a review. Oncologist 11(8): 693–97.

TECHNICAL MANUAL

GyneCult™ Fallopian Tube Organoid Medium

TOLL-FREE PHONE 1 800 667 0322
PHONE +1 604 877 0713
INFO@STEMCELL.COM
TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT WWW.STEMCELL.COM

DOCUMENT #10000024527 VERSION 00 MAR 2024