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# Fallopian Tube Epithelial Cell Culture

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The purpose of this protocol is to grow primary fallopian tube epithelial cells as 2D culture.

This protocol explains the method of culturing and maintaining fallopian tube epithelial cells derived from processing human fallopian tube tissue.

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Confluent cells are contact growth inhibited. Ideal conditions for performing cell culture work (ie. Drug treatments, cell cycle analysis, growth analysis, etc) should be ~70-80% confluence.

1.Freshly isolated FTE cells should be plated on Primaria plates at the following densities:

| A | B |
|---|---|
| Plate Size | Cell Number (Cells) |
| 12 well | 2000 |
| 6 well | 5000 |
| 60 mm | 250000-400000 |
| 100 mm | 600000-1000000 |

2. Primaria plates increase adhesion of cells to plate and promote an optimal substrate. The plate should be coated with collagen before plating the cells.
3. Cells should be cultured for 3-5 days with USG media, full media change every 48hrs, until the density is 70-80%.

| A | B |
|---|---|
| Plate Size | Tissue Culture Media Volume (mL) |
| 24 well | 0.5 |
| 12 well | 1 |
| 6 well | 2 |
| 60 mm | 4 |
| 100 mm | 8 |



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**Ultroser G (USG) (Pall Life Sciences 15950-017)**

This is a serum substitute to grow the primary FTSEC line, is supplied as a lyophilized powder and must be reconstituted before use. Add 20 mL of sterile water to one bottle of USG and wait until the material is fully dissolved (this may take up to 20 minutes) at room temperature (RT). Store unused reconstituted USG at -20°C for up to 6 weeks. Do not freeze and thaw more than once and may be filtered (0.22 µm).

**USG medium**

DMEM/F12 (Corning 10-092-CV) + 2% USG + 1% Penicillin/ Streptomycin (P/S):

Under sterile conditions, combine 485mL DMEM/F12 + 10mL of reconstituted USG + 5mL of P/S (P/S is 1%)

**Freezing medium**

Complete growth medium (USG medium) with 50% FBS and 10% DMSO.

DMEM 1X + 20% FBS + 1% P/S + 10% DMSO

**Collagen**

Collagen 1 – Rat tail (Gibco A1048301)

1 mL of collagen to 200 mL of PBS (Lonza 17-516F) + 1% P/S.

**Wash solution**

PBS + 1% P/S

**Trypsin Neutralizing solution (TNS)**

PBS + 2% FBS + 1% P/S

Refer to SDS (Safety Data Sheet) for hazards and safety warnings.

- All cell culture should be performed under sterile conditions in a biological safety cabinet (BSC)
- All personal protective equipment (PPE) should be used
- Use a solution of 70% EtOH (70% ethanol + 30% MilliQ $H_2O$) to disinfect surface and hands prior to working with cells
- All reagents should be at room temperature (20-22°C, 69-72°F). Do not warm using water bath.
- Obtain patient consent and approval from Research Ethics Board (REB) or Institutional Review Board (IRB) prior to commencing cell culture.



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## Plating cells from pellets (ie. Passaging cells)

1 Cells can be grown after being pelleted in Step 1.8. This is called passaging the cells. The purpose of this step is expand cells onto a larger surface area for growth.

1.1 Prepare cell culture plate by treating cell culture plate with collagen (1mL of collagen for a 100 mm plate)

1.2 Coat entire surface of plate with collagen and aspirate remaining collagen

1.3 Add cell culture media to plate and let sit for 5 mins or more in BSC or until ready to use.

| Plate Size | Tissue Culture Media Volume (mL) |
|---|---|
| 24 well | 0.5 |
| 12 well | 1 |
| 6 well | 2 |
| 60 mm | 4 |
| 100 mm | 8 |

1.4 Add 1 mL of cell culture media to 15mL conical tube with pellet (there should be no supernatant at this point)

1.5 Gently resuspend pellet by pipetting up and down until pellet has dissociated

1.6 Take entire volume and dispense into centre of plate while gently rocking the plate back and forth (this helps to evenly distribute cells onto plate)

1.7 Place plate into incubator (37°C, 5% $CO_2$)



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Dissociating cells from cell culture plate

## 2 Dissociate FTE cells from tissue culture plates

### 2.1 Wash cells with wash solution (PBS +1% P/S)

### 2.2 Aspirate wash solution with an autoclaved borosilicate glass pipette

### 2.3 Add 0.25% Trypsin EDTA to each plate

| Plate Size | Trypsin Volume (mL) |
|---|---|
| 12 well | 0.25 |
| 6 well | 0.5 |
| 60 mm | 1 |
| 100 mm | 2 |

### 2.4 Place plate in incubator (37°C, 5% $CO_2$) for 5 minutes or until cells detach (visualize under inverted lab microscope to confirm detachment)

### 2.5 Remove plate from incubator and add double the volume of TNS to plate (ie 2 mL TNS : 1mL Trypsin). Gently pipette media up and down to collect all cells in pipette.

### 2.6 Transfer to 15 mL conical tube of choice

### 2.7 Centrifuge at 1100 rpm for 5 min

### 2.8 Aspirate supernatant and discard without disturbing pellet



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2.9 Cells are now ready for: Passaging onto new plate; Freezing for future use or DNA/RNA/Protein extraction.

## Plate previously frozen cells

3 Frozen cells should be stored at -80C for short term storage (no more than 1 month) or in Liquid N2 for long term storage).

3.1 ⚠

Take cells out of storage and warm to liquid by holding in the palm of hand (this process should be as quick as possible)

3.2 During thaw, add TNS to 15 mL labelled conical tube

3.3 Add thawed cells using pipette to 15 mL conical tube containing TNS. *For primary fallopian tube cells previously frozen, thaw cryo-vial rapidly into 5ml pre-warmed USG media.

3.4 Centrifuge cells in centrifuge at 1100 rpm for 5 minutes

3.5 Aspirate supernatant

3.6 Re-suspend cells in cell culture media and add to prepared cell culture plate

## Freezing cells

4 Cells that have been pelleted can be resuspended in freezing media and placed directly at -80°C or Liquid N2



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