GyneCult™ Endometrial Organoid Medium

Cell culture medium for expansion and differentiation of endometrial organoids

Catalog #100-2147 1 Kit

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

GyneCult™ Endometrial Organoid Medium (EOM) is a serum-, phenol red-, and sex steroid hormone-free medium for culturing freshly isolated or cryopreserved primary human endometrial epithelial cells in matrix-embedded droplet organoid cultures. Single cells seeded into GyneCult™ EOM cultures grow into cystic organoids (50 - 500 µm in diameter) within 10 days, at which point they are ready for passage by dissociation into single-cell suspensions and subculturing. Cultures can be passaged at least 5 times for a minimum of 10 - 12 doublings.

GyneCult™ EOM can be supplemented with varying levels of estrogen and/or progesterone during culture to adequately model the menstrual cycle. Cultures derived in medium supplemented with estrogen to simulate the proliferative phase express the endometrial stem cell transcription factor SRY-box transcription factor 9 (SOX9), as well as the expected lineage markers (e.g. paired box gene 8, PAX8). These cells persist for at least 7 days in estrogen-containing conditions. The same proliferative-phase organoids can be subsequently differentiated into secretory-phase organoids with supplementation of estrogen and progesterone after day 7 of culture. Cells in these differentiated organoids assume a columnar morphology, lose the expression of SOX9, and display apical concentration of the secretory protein progestagen-associated endometrial protein (PAEP), as would be consistent with apocrine secretion, a hallmark function of secretory endometrial cells in the secretory phase. Furthermore, cells with bundles of beating cilia (stained for acetylated-alpha tubulin) are readily observable in organoids in both the proliferative and secretory phases.

Morphology, lineage makeup, and growth kinetics remain consistent from early to late passages. Cells can be cryopreserved and thawed as needed without growth lag, phenotypic changes, or diminished performance.

NOTE: Primary endometrial cells are required to generate endometrial organoids. To prepare single-cell suspensions of endometrial tissue, follow the same instructions for fallopian tube tissues in the Technical Manual: GyneCult™ Fallopian Tube Organoid Medium (Document #10000024527), available at www.stemcell.com, or contact us to request a copy.

Product Information

The following components are sold as a complete kit (Catalog #100-2147) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
GyneCult™ Endometrial Organoid Basal Medium	100-2144	90 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
GyneCult™ Endometrial Organoid Medium 10X Supplement	100-2145	10 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
GyneCult™ Endometrial Organoid Medium 100X Supplement	100-2146	1 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
17 beta-Estradiol	100-1647
24-Well Flat-Bottom Plate, Non-Treated	38042
Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix, Phenol Red-free, LDEV-Free	Corning 356231
CryoStor® CS10	07930
Fetal Bovine Serum	100-0180
HBSS with 10 mM HEPES, Without Phenol Red	37150
Trypsin-EDTA (0.25%)	07901
Y-27632 (Dihydrochloride)	72302
Optional: Antibiotic(s)/Antimycotic(s)	--

Preparation of Media

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

1. Thaw GyneCult™ Endometrial Organoid Medium 10X Supplement and GyneCult™ Endometrial Organoid Medium 100X Supplement at room temperature (15 - 25°C) for 1 hour. Mix thoroughly and store on ice.
2. Aliquot 89 mL of GyneCult™ Endometrial Organoid Basal Medium into a sterile container.

   NOTE: If using antibiotics, reduce the volume of Basal Medium to accommodate antibiotic volume.
3. Add 10 mL of GyneCult™ Endometrial Organoid Medium 10X Supplement to the Basal Medium aliquot.

   NOTE: If not using 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™ Endometrial Organoid Medium 100X Supplement.

   NOTE: If not using 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. Optional: Add 17 beta-Estradiol to a final concentration of 0.2 nM.

   NOTE: Adding 17 beta-Estradiol is recommended if organoids will be passaged. Omit this step if hormone-free medium is desired and organoids will not be passaged.
6. Optional: Add antibiotic(s)/antimycotic(s) as desired.

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

Protocol Diagram

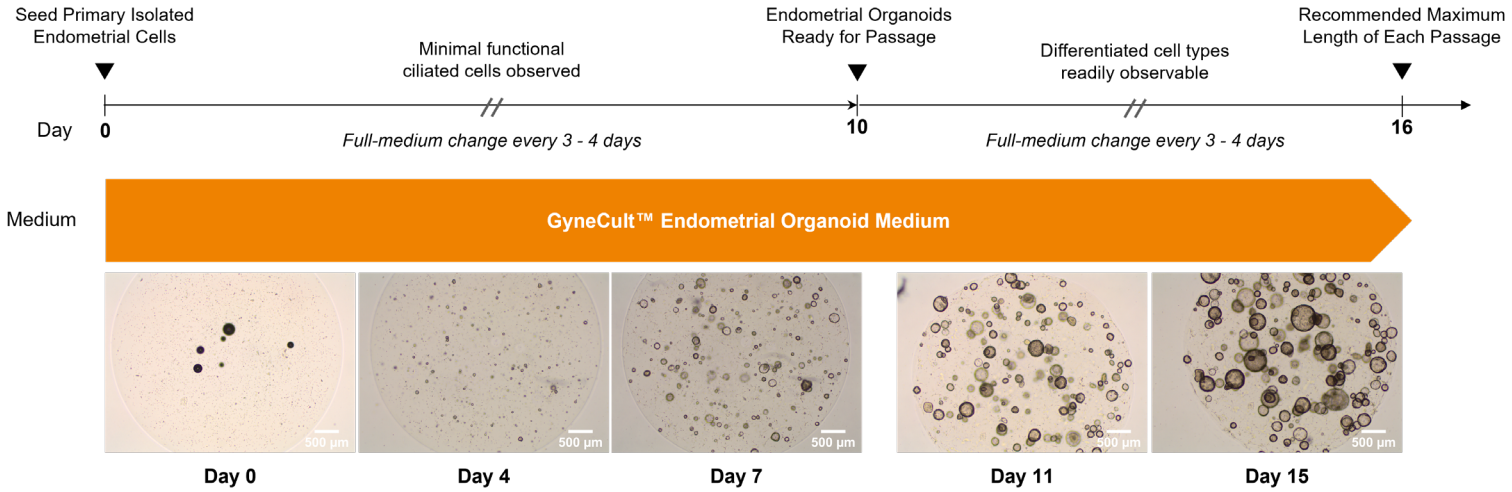

Figure 1. Protocol Diagram for Organoid Generation from Primary Endometrial Cells Using GyneCult™ Endometrial Organoid Medium and Representative Images of Expected Organoid Morphology

Directions for Use

A. Initiation of Human Endometrial Organoid Cultures

The following instructions are for generating endometrial organoids from primary endometrial cells. Prior preparation of single-cell suspensions of endometrial tissue is required. For full instructions, refer to sections 5.0 and 6.1 of the Technical Manual: GyneCult™ Fallopian Tube Organoid Medium (Document #10000024527), available at www.stemcell.com, or contact us to request a copy. Instructions for fallopian tube tissue are applicable for endometrial tissue.

1. Prepare human endometrial single-cell suspensions from primary dissociated tissue or from cryopreserved vials of dissociated tissue.
2. Thaw the required volume of Corning® Matrigel® on ice (20 µL per dome).
   NOTE: The recommended seeding density is 4000 cells per dome and up to 3 domes per well in a 24-well non-tissue culture-treated plate.
3. Aliquot the desired number of cells into sterile microcentrifuge tubes, up to 1 x 10^5 cells per tube.
   NOTE: If cell numbers exceed stated numbers, use additional sterile microcentrifuge tubes at the same ratio of cells per tube.
4. Centrifuge tubes at 430 x g for 5 minutes at 2 - 8°C.
5. Keep tubes on ice after centrifugation.
6. Use a 24-well non-tissue culture-treated plate and process one tube/plate at a time as follows:
   a. Working quickly and minimizing contact between fingertips and the tube, aspirate supernatant and add 20 µL of Corning® Matrigel® to the tube for each dome to be seeded from that tube. Pipette up and down 8 - 10 times quickly but gently to create a uniform suspension, avoiding bubbles by dispensing only to the first stop of the pipettor.
      NOTE: Pipette tips can be cooled when working with Matrigel® to help minimize premature solidifying.
   b. Using a 20 µL pipettor, add 20 µL of Matrigel®-cell suspension to the center of the well if plating 1 dome per well, or off-center if seeding 2 - 3 domes per well (ensuring that each dome is equidistant from the center of the well). Avoid generating bubbles by dispensing only to the first stop of the pipettor.
   c. Repeat step 6b 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 dome(s).
   e. Incubate the plate at 37°C for 15 - 20 minutes.
      NOTE: If preparing more than one microcentrifuge tube of thawed cells, step 6a can be started for the next tube during this incubation period.
   f. Prepare a sufficient volume of complete GyneCult™ EOM for the number of wells plated (500 µL per well). 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 against the wall of the well to avoid disturbing the dome(s).
7. Place the lid on the culture plate. Carefully transfer the plate to the 37°C and 5% CO_2 incubator.

B. Maintenance of Human Endometrial Organoid Cultures

1. 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 complete medium (without Y-27632 [Dihydrochloride]).

   NOTE: Y-27632 (Dihydrochloride) can be kept in the medium for the duration of the culture if desired.
2. Perform a full-medium change every 3 - 4 days.
3. Passage cultures between day 10 and day 14 after seeding (refer to section C below).

   NOTE: Cultures should be passaged or harvested by day 16.

C. Passaging Human Endometrial Organoids

1. Prepare wash buffer (WB; HBSS with 10 mM HEPES, Without Phenol Red + 2% Fetal Bovine Serum).
2. Warm 1 mL of Trypsin-EDTA (0.25%) to 37°C for each well to be passaged. Process a maximum of 8 wells at a time.
3. Without touching the dome(s), aspirate culture medium completely.
4. Add 1 mL of Trypsin-EDTA (0.25%) per well.
5. For each well, pipette up and down 10 times, forcefully and quickly to shear large Matrigel® aggregates. Avoid bubble formation by dispensing only to the first stop of the pipettor.
6. Incubate the plate at 37°C for 10 minutes.

   NOTE: if processing more than one plate, steps 3 - 6 can be initiated for a new plate during this incubation step.
7. For each well, aliquot 1 mL of cold WB into a 15 mL conical tube. Keep tubes on ice.
8. Using a 1 mL pipettor, triturate each well again, 10 times. Incubate at 37°C for 10 minutes.
9. Triturate again as in step 8 and transfer dissociated cells to the conical tube containing 1 mL of WB. Triturate 5 times to mix thoroughly.
10. Wash the emptied well with 1 mL of WB 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 3 - 5 times to mix. Keep the tube on ice.
11. Repeat steps 9 - 10 for the remaining wells, processing one well at a time.
12. Centrifuge the tube(s) at 430 x g for 5 minutes at 2 - 8°C.
13. Aspirate the supernatant and resuspend in a minimum volume of 250 - 500 µL of complete medium or WB. Mix by pipetting 5 - 8 times.
14. Perform a live cell count and aliquot desired number of cells in sterile microcentrifuge tubes, up to 1 x 10^5 cells per tube (recommended seeding density is 4000 cells per dome).

    NOTE: If seeding more cells, use additional sterile microcentrifuge tubes at the same ratio of maximum cells per tube.
15. To seed into the next passage, refer to section A above.

    NOTE: Cells can be cryopreserved at this stage using CryoStor® CS10 at 0.5 - 2 x 10^6 cells per mL. For instructions on cryopreserving and thawing cells, refer to the Product Information Sheet for CryoStor® CS10 (Document #10000000383), available at www.stemcell.com, or contact us to request a copy.

Related Products

For related products, including specialized culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com or contact us at techsupport@stemcell.com.