IntestiCult™ Organoid Growth Medium (Human)

Cell culture medium for establishment and maintenance of human intestinal organoids

Catalog #06010 100 mL

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

IntestiCult™ Organoid Growth Medium (Human) is a complete cell culture medium for efficient establishment and long-term maintenance of intestinal organoids derived from human intestinal crypts. Intestinal organoids provide a convenient in vitro organotypic culture system for studying the intestinal epithelium. The organoids incorporate a functional lumen enclosed by a polarized intestinal epithelial cell layer. Applications of intestinal organoid cultures include studying the development and function of intestinal epithelium, modeling intestinal diseases, and screening molecules for both efficacy and toxicity in an intestinal model. Intestinal organoid cultures can also be used for investigation of adult stem cell properties and for regenerative therapy approaches.

Product Information

The following components are sold as a complete kit (Catalog #06010) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
IntestiCult™ OGM Human Component A*	06011	50 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
IntestiCult™ OGM Human Component B	06012	50 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

*This component contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
DMEM/F-12 with 15 mM HEPES	36254
25% Bovine serum albumin (BSA) in phosphate-buffered saline (PBS)	---
Corning® Matrigel® Matrix, Growth Factor Reduced (GFR), Phenol Red-Free	Corning 356231
D-PBS (Without Ca++ and Mg++)	37350
Gentle Cell Dissociation Reagent	07174
Costar® 24 Well Flat-Bottom Plate, Tissue Culture-Treated	38017
Falcon® 70 µm Cell Strainer	Corning 352350
Y-27632	72302

Preparation of Reagents

A. COMPLETE INTESTICULT™ ORGANOID GROWTH MEDIUM (HUMAN)

Use sterile techniques to prepare complete IntestiCult™ Organoid Growth Medium (Component A + Component B). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw Components A & B at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C for up to 3 months. After thawing the aliquots, use immediately. Do not re-freeze.
2. Add 50 mL of Component B to 50 mL of Component A. Mix thoroughly.

   NOTE: If not used immediately, store complete medium at 2 - 8°C for up to 1 week.
3. Add desired antibiotics immediately before use (e.g. 50 µg/mL gentamicin or 100 units [100 µg/mL] penicillin/streptomycin).

B. DMEM + 1% BSA

Use sterile techniques to prepare DMEM + 1% BSA. The following example is for preparing 50 mL of DMEM + 1% BSA. If preparing other volumes, adjust accordingly.

1. Add 2 mL of 25% BSA to 48 mL of DMEM/F-12 with 15 mM HEPES in a 50 mL conical tube (e.g. Catalog #38010).
2. Mix well by inversion. Place on ice.

   NOTE: If not used immediately, store at 2 - 8°C for up to 6 months.

Directions for Use

Please read the entire protocol before proceeding.

Use sterile techniques when performing the following protocols.

A. ISOLATION OF HUMAN COLONIC CRYPTS FROM BIOPSIES

1. Thaw 100 µL of Matrigel® on ice.

   NOTE: This is sufficient for plating up to 4 culture domes. Depending on the crypt count (section B step 1), a different amount of Matrigel® may be required.
2. Place the following reagents on ice: D-PBS (Without Ca++ and Mg++) and DMEM + 1% BSA (Preparation of Reagents, section B).
3. Warm a tissue culture-treated 24-well plate in a 37°C incubator for at least 2 hours.
4. In a 15 mL conical tube (e.g. Catalog #38009), wash the tissue sample with 10 mL of ice-cold PBS. Allow the tissue to settle by gravity (~ 5 seconds), then aspirate supernatant.
5. Repeat step 4, leaving 1 mL of supernatant in the tube.
6. Transfer the tissue and remaining supernatant to a 1.5 mL microcentrifuge tube using a 1 mL pipettor.
7. Using sterile scissors, thoroughly mince the tissue into the smallest pieces possible. Transfer the tissue fragments to a new 15 mL conical tube using a 1 mL pipettor. Rinse the microcentrifuge tube with PBS and add the rinse to the tissue fragments.
8. Allow the tissue fragments to settle by gravity (~5 seconds), then aspirate supernatant.
9. Add 10 mL of Gentle Cell Dissociation Reagent (GCDR). Incubate on ice on a rocking platform set at medium speed (~40 rpm) for 30 minutes.
10. Centrifuge at 290 x *g* for 5 minutes. Aspirate supernatant.

NOTE: For the remainder of the protocol, pre-wet pipette tips with DMEM + 1% BSA before manipulating the tissue sample. This prevents crypts from sticking to the wall of the pipette tip.

11. Add 1 mL of ice-cold DMEM + 1% BSA. Vigorously pipette up and down 20 times with a 1 mL pipettor to remove crypts from tissue.

    NOTE: Avoid touching the side/bottom of the tube with the pipette tip.
12. Using a 1 mL pipettor, pass the contents of the tube through a 70 µm cell strainer (tilted on its side) into a new 15 mL conical tube. Rinse the original tube with 1 mL of DMEM + 1% BSA and pass through the strainer into the tube. Proceed to section B for organoid culture.

B. ORGANOID CULTURE FROM ISOLATED HUMAN COLON CRYPTS

1. Determine the total number of crypts in the sample (from section A) as follows:
   a. Place 3 x 10 µL aliquots of the sample on an appropriate counting surface (e.g. glass slide or one well of a 6-well plate).
   b. Using an inverted microscope, count the crypts in each aliquot.
   c. Determine the average number of crypts in the 3 aliquots, then multiply by 200 to determine the total number of crypts in the 2 mL sample.
   d. Determine how many culture domes can be plated at 1000 crypts per dome.

   Example: *Aliquot 1: 18 crypts*
   Aliquot 2: 23 crypts
   Aliquot 3: 19 crypts
   Average: 20 crypts x 200 = 4000 crypts total
   This is sufficient for 4 culture domes containing 1000 crypts each.

   NOTE: 1000 crypts/dome will result in 150 - 200 mature organoids.

2. Centrifuge the sample at 200 x *g* for 5 minutes. Aspirate all except 100 µL of supernatant.

NOTE: The following steps are for plating 4 x 50 µL culture domes containing 1000 crypts each. If fewer or additional culture domes are required based on the counts in step 1, adjust the volume of Matrigel® and DMEM + 1% BSA to give a 1:1 final mixture (e.g. for 8 x 50 µL culture domes, add 200 µL Matrigel® and 100 µL DMEM + 1% BSA to the sample tube).

3. Remove the 24-well plate from the 37°C incubator. Pre-wet a 200 µL pipette tip with DMEM + 1% BSA.
4. Add 100 µL of Matrigel® to the sample tube. Pipette up and down 10 times to thoroughly resuspend the pellet. Avoid introducing bubbles.
5. Using a pre-wetted 200 µL pipette tip, draw up 50 µL of the Matrigel®-crypt suspension and add to 1 of the 8 central wells of a 24-well tissue culture-treated plate as follows:
   a. Hold the pipette vertically over the center of the well. Bring the pipette tip near to but not in contact with the floor of the well.
   b. Slightly depress the plunger until a droplet is visible on the end of the pipette tip.
   c. Slowly lower the pipette until the droplet touches the floor of the well.
   d. Gently dispense (only to the first stop on the pipette) the remaining volume while lifting the pipette away from the well.

   NOTE: Work quickly to plate the Matrigel®-crypt suspension within ~60 seconds of removing it from ice.
6. Repeat step 5 until all of the Matrigel®-crypt suspension is dispensed.
7. Carefully transfer the plate to a 37°C incubator. Incubate at 37°C for 10 minutes to allow domes to solidify. Do not disturb the domes.
8. Prepare 3 mL of IntestiCult™ Organoid Growth Medium (Preparation of Reagents, section A) at room temperature (15 - 25°C).
   For primary culture (organoids have not yet been passaged), add 10 µL of 3 mM Y-27632 (10 µM final concentration). Mix thoroughly.

   NOTE: Each culture dome requires 750 µL of IntestiCult™ Organoid Growth Medium; 3 mL of medium is sufficient for 4 culture domes. If preparing a different number of culture domes, adjust volume of medium accordingly.
9. Add 750 µL of IntestiCult™ Organoid Growth Medium (+ Y-27632 for primary culture) to each well by pipetting the medium gently down the wall of the well. Do not pipette directly onto the domes.
10. Add sterile PBS to unused wells.
11. Place the lid on the culture plate and incubate at 37°C and 5% CO_2.
12. Every 2 days, perform a full medium change with IntestiCult™ Organoid Growth Medium (Y-27632 is not required).
13. Proceed to section C for passaging.

C. PASSAGING HUMAN INTESTINAL ORGANOIDS

For primary cultures, passage after 7 - 14 days. For previously passaged organoids, passage every 7 - 10 days. Larger cystic or budded organoids will result in a higher yield of viable fragments than smaller, dark, collapsed, or overly-budded organoids.

1. Warm a 24-well tissue culture-treated plate in a 37°C incubator for at least 2 hours.
2. Prepare IntestiCult™ Organoid Growth Medium (Human) and warm to room temperature (15 - 25°C).

   NOTE: For each well to be passaged, 750 µL of medium will be required.
3. Thaw Matrigel® on ice; for each well to be plated, 25 µL of Matrigel® will be required.
4. Place DMEM + 1% BSA on ice.
5. Carefully remove and discard medium from each well to be passaged, without disturbing the Matrigel® dome.

6. Add 1 mL of room temperature (15 - 25°C) GCDR on top of the exposed dome in each well. Incubate for 1 minute at room temperature.
7. Pre-wet a 1 mL pipette tip with GCDR; use this pipette tip to thoroughly scrape the Matrigel® dome free of the well floor. Pipette the GCDR in the well up and down 2 - 3 times to break up the dome and the organoids. Ensure all pieces of Matrigel® have been rinsed free of the plate.

   NOTE: When pipetting up and down, avoid touching the bottom of the well with the pipette tip.
8. Using the same pipette tip, transfer the organoid mixture to a 15 mL conical tube.
9. Add 1 mL of GCDR to the newly emptied well. Using a pipette tip pre-wetted with GCDR, pipette the GCDR up and down 2 - 3 times to rinse the well. Transfer the contents of the well to the 15 mL conical tube from step 8.
10. Repeat steps 7 - 9 for each well to be passaged.
11. Incubate the tubes at room temperature (15 - 25°C) on a rocking platform set at medium speed (~40 rpm) for 10 minutes.
12. Centrifuge the tubes at 290 x *g* for 5 minutes at 2 - 8°C. Gently pour off and discard the supernatant.
13. Add 1 mL of ice-cold DMEM + 1% BSA to each tube. Using a pre-wetted 1 mL pipette tip, resuspend organoids by pipetting up and down vigorously 15 times.

    NOTE: Avoid touching the side/bottom of the tube with the pipette tip.
14. Using a 1 mL pipettor, pass the contents of the tube through a 70 µm cell strainer (tilted on its side) into a new 15 mL conical tube. Rinse the original tube with 1 mL of DMEM + 1% BSA and pass through the strainer into the tube.

For subsequent steps in the passaging protocol, refer to section B.

Notes and Tips

- Complete disruption of organoids will result in single-cell cultures that will grow in IntestiCult™ Organoid Growth Medium, but will take longer to reach maturity; organoids derived from single cells will have a predominantly cystic morphology.
- Matrigel® is temperature-sensitive and will polymerize at room temperature within a few minutes; keep Matrigel® on ice at all times to prevent polymerization prior to plating.

STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485. PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED.

Copyright © 2018 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, and IntestiCult are trademarks of STEMCELL Technologies Canada Inc. Matrigel, Costar, Falcon, and Corning are registered trademarks of Corning Incorporated. All other trademarks are the property of their respective holders. This product was developed under a license to intellectual property owned by The Hubrecht Organoid Technology Foundation ("the HUB"). This product is sold for research use only. Purchase of this product does not include the right to use this product for growing organoids for drug screening aiming for commercial gain or for other commercial purposes. Purchasers wishing to use the product for purposes other than research use should contact the HUB to obtain a further license (an "Organoid-Growth License"). Purchasers may apply for an Organoid-Growth License from the HUB and such license will not be unreasonably withheld by the HUB. While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.