IntestiCult™-SF Organoid Growth Medium (Human)

Serum-free and conditioned medium-free cell culture medium for establishment and maintenance of human intestinal organoids

Catalog #100-0340 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™-SF Organoid Growth Medium (Human) is a complete cell culture medium for efficient establishment and long-term maintenance of organoids derived from human intestinal crypts. IntestiCult™-SF is a serum-free and conditioned medium-free formulation.

Intestinal organoids provide a convenient in vitro organotypic culture system for studying the intestinal epithelium. Isolated intestinal crypts rapidly form complex organoids when cultured in IntestiCult™-SF. The organoids incorporate a functional lumen enclosed by a polarized epithelial cell layer containing known cell types of the adult intestinal epithelium. Maturing intestinal organoids establish protruding buds, resembling intestinal crypts of the in vivo tissues. IntestiCult™-SF supports the proliferation of intestinal epithelial stem cells from patient's biopsies and expansion of organoid cultures derived from fresh tissue or from already established frozen stocks.

Applications of intestinal organoid cultures include studying the development and function of intestinal epithelium, modeling intestinal diseases, and performing targeted molecule screens. Intestinal organoid cultures can also be used for investigation of adult stem cell properties and regenerative therapy approaches, and can be further differentiated using IntestiCult™ Organoid Differentiation Medium (Human; Catalog #100-0214).

Properties

Storage: Store at -20°C.

Shelf Life: Stable for 2 years from date of manufacture (MFG) on label.

This product contains material derived from human plasma. Donors have been tested and found negative for hepatitis B surface antigen (HBsAg), HIV-1 antibodies, and/or HIV-1 antigen. 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® Growth Factor Reduced (GFR) Basement Membrane Matrix, Phenol Red-Free, LDEV-free	Corning 356231
D-PBS (Without Ca++ and Mg++)	37350
Gentle Cell Dissociation Reagent	100-0485
Costar® 24-Well Flat-Bottom Plate, Tissue Culture-Treated	38017
70 µm Reversible Strainer, Small	27216
Y-27632 (Dihydrochloride)	72302
15 mL and 50 mL conical tubes	e.g. 38009 and 38010
1.7 mL microcentrifuge tubes	e.g. 38089

Preparation of Reagents

A. INTESTICULT™-SF ORGANOID GROWTH MEDIUM (IntestiCult™-SF)

Use sterile technique to prepare IntestiCult™-SF.

1. Thaw IntestiCult™-SF Organoid Growth Medium (Human) at room temperature (15 - 25°C) or at 2 - 8°C overnight. Invert the bottle to mix thoroughly.

   NOTE: If not used immediately, store at 2 - 8°C for up to 2 weeks. Alternatively, aliquot and store at -20°C for up to 3 months. Do not exceed the shelf life of the medium. After thawing the aliquots, use immediately. Do not re-freeze.
2. Immediately before use, add desired antibiotics (e.g. 50 µg/mL gentamicin or 100 units [100 µg/mL] penicillin/streptomycin).

B. DMEM + 0.1% BSA

Use sterile technique to prepare DMEM + 0.1% BSA. The following example is for preparing 50 mL of DMEM + 0.1% BSA. If preparing other volumes, adjust accordingly.

1. Add 0.2 mL of 25% BSA in PBS to 49.8 mL of DMEM/F-12 with 15 mM HEPES in a 50 mL conical tube.
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 technique when performing the following protocols.

A. ISOLATION OF HUMAN INTESTINAL CRYPTS FROM BIOPSY SAMPLES

1. Warm a tissue culture-treated 24-well plate in a 37°C incubator for at least 2 hours.
2. Thaw 100 µL of Corning® Matrigel® on ice.

   NOTE: This is sufficient for plating up to 8 culture domes. Depending on the crypt count (section B step 1), a different amount of Matrigel® may be required.

   NOTE: Matrigel® is temperature-sensitive and should be kept on ice at all times. It will start to polymerize at room temperature within a few minutes and will become more difficult to manipulate.
3. Prepare IntestiCult™-SF (Preparation of Reagents, section A). Warm an aliquot to room temperature (15 - 25°C).
4. Place the following reagents on ice: D-PBS (Without Ca++ and Mg++), Gentle Cell Dissociation Reagent (GCDR), and DMEM + 0.1% BSA (Preparation of Reagents, section B).
5. In a 15 mL conical tube, wash the tissue sample with 10 mL of ice-cold D-PBS. Allow the tissue to settle by gravity (~ 5 seconds), then remove and discard supernatant.
6. Repeat step 5, leaving 1 mL of supernatant in the tube.
7. Using a 1 mL pipettor, transfer the tissue and remaining supernatant to a 1.7 mL microcentrifuge tube.
8. 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 D-PBS and add the rinse to the tissue fragments.
9. Centrifuge at 290 x *g* for 5 minutes. Aspirate the supernatant.
10. Add 10 mL of ice-cold GCDR. Incubate on ice on a rocking platform set at medium speed (~40 rpm) for 30 minutes.
11. Centrifuge at 290 x *g* for 5 minutes. Aspirate the supernatant.

NOTE: For the remainder of the protocol, pre-wet pipette tips with DMEM + 0.1% BSA before manipulating the tissue sample. This prevents crypts from sticking to the wall of the pipette tip.

12. Add 1 mL of ice-cold DMEM + 0.1% 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.
13. Using a 1 mL pipettor, pass the contents of the tube through a 70 µm strainer into a new 15 mL conical tube. Rinse the original tube with 1 mL of DMEM + 0.1% BSA and pass through the strainer into the tube. Proceed to section B for organoid culture.

B. ORGANOID CULTURE FROM ISOLATED CRYPTS

1. Determine the total number of crypts in the 2 mL 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. Calculate the average number of crypts per 10 µL aliquot, then determine the total number of crypts in the 2 mL sample.

Example:

Aliquot 1: 18 crypts; Aliquot 2: 23 crypts; Aliquot 3: 19 crypts

Average: 20 crypts/10 µL

Total number of crypts: 20 crypts/10 µL x 2000 µL = 4000 crypts

d. Determine how many culture domes can be plated at 1000 crypts per dome (e.g. 4000 crypts 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. Gently remove and discard most of the supernatant. A small volume of supernatant (10 - 20 µL) can be left on top of the pellet. Place tube on ice.

NOTE: The following steps are for plating 4 x 50 µL culture domes containing 1000 crypts each. If an alternate number of culture domes are required based on the counts in step 1, adjust the volume of Matrigel® and DMEM + 0.1% BSA to give a 1:1 final mixture.

3. Add 80 - 90 µL of DMEM + 0.1% BSA to the pellet to resuspend. The total volume of organoid suspension should be 100 µL.
4. Add 100 µL of Matrigel® to the sample tube. Pipette up and down 10 times to thoroughly resuspend the pellet. Avoid introducing bubbles. Keep the tube on ice.
5. Remove the 24-well plate from the 37°C incubator (section A step 1). Pre-wet a 200 µL pipette tip with DMEM + 0.1% BSA.
6. Using a pre-wetted 200 µL pipette tip, draw up 50 µL of the Matrigel®-crypt suspension and add to one of the eight central wells of a 24-well tissue culture-treated plate as follows:
   a. Hold the pipettor 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 pipettor until the droplet touches the floor of the well.
   d. Gently dispense (only to the first stop on the pipettor) the remaining volume while lifting the pipettor away from the well.

   NOTE: Work quickly to plate the Matrigel®-crypt suspension within ~60 seconds of removing it from ice.
7. Repeat step 6 until all of the Matrigel®-crypt suspension is dispensed.
8. Carefully transfer the plate to a 37°C incubator. Incubate for 20 minutes to allow domes to solidify. Do not disturb the domes.
9. For primary culture (organoids have not yet been passaged), add Y-27632 (Dihydrochloride) to 2 mL of IntestiCult™-SF to a final concentration of 10 µM (e.g. 2 µL of 10 mM Y-27632 [Dihydrochloride] stock solution). Mix thoroughly.

   NOTE: Each culture dome requires 500 µL of medium; 2 mL of medium is sufficient for 4 culture domes. If preparing a different number of culture domes, adjust volume of medium accordingly.
10. Add 500 µL of IntestiCult™-SF (+ Y-27632 [Dihydrochloride] for primary culture) to each well by pipetting the medium gently down the wall of the well. Do not pipette directly onto the domes.
11. Add sterile D-PBS to each corner well of the plate. Place the lid on the plate and incubate at 37°C and 5% CO_2.
12. Every 2 days, perform a full-medium change with 500 µL IntestiCult™-SF (Y-27632 [Dihydrochloride] 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. Warm IntestiCult™-SF to room temperature.

   NOTE: For each well to be passaged, 500 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 GCDR and DMEM + 0.1% BSA on ice.

NOTE: For this protocol, pre-wet pipette tips with DMEM + 0.1% BSA before manipulating organoids. This prevents organoids from sticking to the wall of the pipette tip.

5. Check the organoid culture under a microscope and estimate the split ratio to be applied for passaging. The split ratio will depend on the organoid line and the segment of the gut from which the initial biopsy was collected.
6. Carefully remove and discard medium from each well to be passaged, without disturbing the Matrigel® dome.
7. Add 1 mL of ice-cold GCDR on top of the exposed dome in each well. Incubate at room temperature for 1 minute.

8. Pre-wet a 1 mL pipette tip with DMEM + 0.1% BSA; 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.
9. Using the same pipette tip, transfer the organoid mixture to a 15 mL conical tube.
10. Add 1 mL of GCDR to the newly emptied well. Using a pipette tip pre-wetted with DMEM + 0.1% BSA, 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 9.
11. Repeat steps 8 - 10 for each well to be passaged. Multiple wells of the same sample can be collected in the same tube.
12. Place the tubes on ice for 10 minutes.
13. Centrifuge the tubes at 290 x *g* for 5 minutes at 2 - 8°C. Gently pour off and discard the supernatant.
14. Add 1 mL ice-cold DMEM to each tube. Using a 1 mL pipette tip pre-wetted with DMEM + 0.1% BSA, resuspend organoids by pipetting up and down vigorously 20 - 25 times.

    NOTE: Avoid touching the side/bottom of the tube with the pipette tip.

    NOTE: The amount of trituration required to achieve good organoid fragmentation depends on the amount and the morphology of organoids. Cystic organoids tend to fragment more easily compared to budded, condensed organoids. Also, if more wells were collected in the same tube, additional pipetting may be needed.
15. Add 3 mL DMEM + 0.1% BSA to each tube.
16. Centrifuge the tubes at 290 x *g* for 5 minutes at 2 - 8°C. Gently remove and discard most of the supernatant. A small volume of supernatant (10 - 20 µL) can be left on top of the pellet. Place tubes on ice.

NOTE: The following steps are for plating 4 x 50 µL domes using a split ratio of 1:4. If an alternate number of culture domes are required based on the number of starting wells and desired passaging ratio, adjust the volume of Matrigel® and DMEM + 0.1% BSA to give a 1:1 final mixture.

17. Add 80 - 90 µL of DMEM + 0.1% BSA to the pellet to resuspend. The total volume of organoid suspension should be 100 µL.
18. Add 100 µL of Matrigel® to the organoid suspension. Mix gently by pipetting up and down 10 times. Avoid introducing bubbles. Keep the tube on ice.
19. Remove the 24-well plate from the 37°C incubator.
20. Using a pre-wetted 200 µL pipette tip, draw up 50 µL of the Matrigel®-organoid suspension and add to one of the eight central wells of a 24-well tissue culture-treated plate as follows:
    a. Hold the pipettor 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 pipettor until the droplet touches the floor of the well.
    d. Gently dispense (only to the first stop on the pipettor) the remaining volume while lifting the pipettor away from the well.

    NOTE: Work quickly to plate the Matrigel®-organoid suspension within ~60 seconds of removing it from ice.
21. Repeat step 20 until all of the Matrigel®-organoid suspension is dispensed.
22. Carefully transfer the plate to a 37°C incubator. Incubate for 20 minutes to allow domes to solidify. Do not disturb the domes.
23. Add 500 µL of room temperature IntestiCult™-SF to each well by pipetting the medium gently down the wall of the well. Do not pipette directly onto the domes.
24. Add sterile D-PBS to each corner well of the plate. Place the lid on the plate and incubate at 37°C and 5% CO_2.
25. Every 2 days, perform a full-medium change with 500 µL of room temperature IntestiCult™-SF.
26. Passage organoids every 5 - 10 days, depending on morphology and culture density.