IntestiCult™ Organoid Growth Medium (Mouse)

Cell culture medium for establishment and maintenance of mouse intestinal organoids

Catalog #06005 1 Kit

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Product Description

IntestiCult™ Organoid Growth Medium (Mouse) is a defined, serum-free cell culture medium for efficient establishment and long-term maintenance of mouse intestinal organoids.These organoids, or “mini-guts”, provide a convenient in vitro organotypic culture system for studying both the small and large intestinal epithelium and associated stem cell dynamics. Organoids grown in IntestiCult™ feature a polarized epithelium that contains all of the known cell types of the adult intestinal epithelium. Individual intestinal crypts rapidly form organoids when cultured in IntestiCult™ Organoid Growth Medium (Mouse). Applications of these cultures include studying the development and function of the normal and tumorigenic intestinal epithelium, modeling intestinal disease, and investigating stem cell properties and regenerative therapy approaches. Organoid culture enables convenient in vitro characterization of a system with strong physiological relevance to the adult intestine.

Product Information

The following components are sold as a complete kit (Catalog #06005) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
IntestiCult™ OGM Mouse Basal Medium	06000	90 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
IntestiCult™ OGM Mouse Supplement 1*	06002	5 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
IntestiCult™ OGM Mouse Supplement 2	06003	5 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.

*This product 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.

None of the above components contain antibiotics.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
D-PBS (Without Ca++ and Mg++)	37350
Gentle Cell Dissociation Reagent	07174
DMEM/F-12 with 15 mM HEPES	36254
Bovine serum albumin (BSA)	---
Corning® Matrigel® Matrix, Growth Factor Reduced (GFR), Phenol Red-Free	Corning 356231
Costar® 24-Well Flat-Bottom Plate, Tissue Culture-Treated	38017
Costar® 6-Well Flat-Bottom Plate, Tissue Culture-Treated	38015
70 µm Reversible Strainer, Large	27260
Falcon® Conical Tubes, 50 mL	38010
Falcon® Conical Tubes, 15 mL	38009
Falcon® Serological Pipettes, 10 mL	38004

Preparation of Complete IntestiCult™ Organoid Growth Medium (Mouse)

Use sterile techniques to prepare complete IntestiCult™ Organoid Growth Medium (Basal Medium + Supplement 1 + Supplement 2 + antibiotics). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw Supplement 1 and Supplement 2 at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.

   NOTE: Once thawed, use immediately.
2. Warm Basal Medium to room temperature (15 - 25°C).
3. Add 5 mL of Supplement 1 and 5 mL of Supplement 2 to 90 mL of Basal Medium. Invert the bottle to mix thoroughly.

   NOTE: If not used immediately, store IntestiCult™ Organoid Growth Medium + Supplements 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 individual components. After thawing the aliquots, do not re-freeze.
4. Immediately before use, add desired antibiotics to fresh or thawed IntestiCult™ Organoid Growth Medium + Supplements (e.g. 50 µg/mL gentamicin or 100 units/100 µg per mL penicillin/streptomycin).

Directions for Use

Please read the entire protocol before proceeding.

Use sterile techniques when performing the following protocols.

For further details, refer to the Technical Bulletin: Intestinal Epithelial Organoid Culture with IntestiCult™ Organoid Growth Medium (Mouse), Document #28223, available at www.stemcell.com or contact us to request a copy.

NOTE: Throughout the protocols below, pre-wet pipettes and pipette tips with D-PBS (Without Ca++ and Mg++) (PBS) before manipulating intestinal pieces or crypts. This prevents the tissue from sticking to the wall of the pipette.

A. ISOLATION OF MOUSE INTESTINAL CRYPTS

1. Thaw 500 µL of Matrigel® on ice.
2. Warm a tissue culture-treated 24-well plate in a 37°C incubator for at least 30 minutes.
3. Sacrifice mouse according to your approved institutional guidelines.
   - Small intestine: Harvest approximately 20 cm of small intestine.
   - Colon: Harvest 3 - 6 cm of colon, a few millimeters below the cecum, per sacrificed mouse.

     NOTE: The following protocol can be performed using the colon from one mouse; however, due to the high degree of variability between new users, use the colons from 2 or 3 mice to ensure a greater seeding density when performing the protocol for the first time.
4. Place the intestine in a 10 cm dish containing 5 mL of cold (2 - 8°C) PBS.
5. Flush the intestine gently with 1 mL of cold (2 - 8°C) PBS by inserting a 1 mL pipette tip into one of the open ends of the intestine.
6. Use small scissors to make a longitudinal incision along the entire length of the intestine. Splay open the intestinal segment and use a pipettor to wash gently with 1 mL of cold (2 - 8°C) PBS. Wash 2 more times for a total of 3 x 1 mL washes.
7. Transfer the intestinal segment to a clean 10 cm dish containing 15 mL of fresh cold (2 - 8°C) PBS. Using forceps, move the segment through the clean buffer to rinse thoroughly.
8. Add 15 mL of cold (2 - 8°C) PBS to a 50 mL conical tube. Using forceps, hold the washed intestine by one end over the tube. Starting from the bottom of the intestine, use scissors to cut the intestine into 2 mm segments, allowing these segments to fall into the buffer in the tube.
9. Use a pre-wetted 10 mL serological pipette to wash the intestinal pieces by pipetting up and down 3 times. Let intestinal pieces settle by gravity and carefully remove supernatant. Add 15 mL of cold (2 - 8°C) PBS. Repeat this wash procedure 15 - 20 times or until supernatant is clear.

   NOTE: When isolating colonic crypts, the supernatant will become clear after 3 - 5 washes. Despite this appearance, wash the colonic pieces 15 times.
10. Remove supernatant and resuspend in 25 mL of Gentle Cell Dissociation Reagent at room temperature (15 - 25°C). Incubate on a rocking platform at 20 rpm for 15 minutes (small intestine) or 20 minutes (colon) at room temperature (15 - 25°C).
11. Let the intestinal pieces settle by gravity for approximately 30 seconds and carefully remove the supernatant.
12. Resuspend the intestinal pieces in 10 mL of cold (2 - 8°C) PBS + 0.1% BSA and pipette up and down 3 times.
13. Let the majority of the intestinal pieces settle to the bottom. Remove the supernatant and pass it through a 70 µm strainer into a 50 mL conical tube. Label the filtrate “Fraction 1” and place on ice.

14. Repeat steps 12 - 13 three times to generate Fractions 2 - 4.

    NOTE: For the colon, an additional 2 fractions may be required. To determine this, add 1 mL of each of Fractions 1 - 4 to separate wells of a 6-well plate and inspect under a bright field microscope at 4X magnification. If Fractions 3 and 4 contain debris, repeat steps 12 - 13 two more times to generate Fractions 5 and 6.
15. Centrifuge the fractions at 290 x *g* for 5 minutes at 2 - 8°C. Carefully pour off and discard the supernatants, leaving the pellet in each tube.
16. Resuspend each pellet in 10 mL of cold (2 - 8°C) PBS + 0.1% BSA.
17. Transfer each suspension to a fresh 15 mL conical tube labeled with the corresponding fraction number.
18. Centrifuge the fractions at 200 x *g* for 3 minutes at 2 - 8°C. Gently pour off and discard the supernatants. Pelleted crypts will remain in the tubes.

B. MOUSE INTESTINAL ORGANOID CULTURE

1. Resuspend each crypt pellet (generated in section A) in 10 mL of cold (2 - 8°C) DMEM/F-12 with 15 mM HEPES.
2. Add 1 mL of each suspension to individual wells of a 6-well plate and assess the quality of the suspensions by using an inverted microscope. Select the suspension(s) that are enriched for intestinal crypts.

   NOTE: Crypts desirable for culture can be of various sizes and resemble small, folded sections of an epithelial monolayer. Fractions 3 and 4 usually have the greatest enrichment for desirable crypts. The 1 mL samples can be added back to their respective fractions.
3. For each selected fraction, count the number of crypts in a 10 µL aliquot using an inverted microscope. Calculate the number of crypts per mL of each fraction (e.g. 15 crypts in 10 µL x 100 = approximately 1500 crypts/mL).
4. From the selected fraction(s), aliquot the crypts into 3 x 15 mL labeled conical tubes in volumes containing approximately 500, 1500, and 3000 crypts. Centrifuge at 200 x *g* for 5 minutes at 2 - 8°C. Remove and discard the supernatant.
5. Add 150 µL of complete IntestiCult™ Organoid Growth Medium at room temperature (15 - 25°C) to each pellet.
6. Add 150 µL of undiluted Matrigel® (thawed in step A1) to each tube and pipette up and down 10 times to resuspend the pellet. Avoid introducing bubbles.
7. Carefully pipette 50 µL of the 500-crypt suspension into each of 4 wells of the pre-warmed 24-well plate (step A2). Hold the pipette tip just above the bottom of the plate and slowly dispense to the first stop of the pipette. The samples should form domes in the middle of each well. Repeat for the 1500-crypt and 3000-crypt suspensions for a total of 12 wells.

   NOTE: Work quickly to plate the 4 wells for each preparation within 30 - 60 seconds of adding Matrigel® to the pellet, as the Matrigel® will begin to solidify.
8. Incubate at 37°C for 10 minutes until the Matrigel® is solidified.

   NOTE: Be careful not to disturb the domes during transfer of the plate to the incubator.
9. Add 750 µL of complete IntestiCult™ Organoid Growth Medium at room temperature (15 - 25°C) to each well by pipetting the medium gently down the wall of the well. Do not pipette directly onto the domed cultures.
10. Add sterile PBS to any unused wells.
11. Place the lid on the culture plate and incubate at 37°C and 5% CO_2.
12. Exchange the culture medium 3 times per week by removing the existing medium and replacing it with 750 µL of fresh, complete IntestiCult™ Organoid Growth Medium at room temperature (15 - 25°C).

    NOTE: Crypts from the small intestine typically start to bud after 2 - 4 days in culture. Colonic crypts typically start to bud after 7 - 10 days in culture, though budding is less defined and in some instances the colon organoid will remain cystic.

C. PASSAGING MOUSE INTESTINAL ORGANOIDS

Mouse organoid cultures from the small intestine should be passaged every 7 - 10 days with an average split ratio of 1:6. Mouse colonic organoid cultures should be passaged every 7 - 10 days, or when the density reaches 150 organoids per well, with an average split ratio of 1:2.

1. Warm IntestiCult™ Organoid Growth Medium + Supplements to room temperature (15 - 25°C) and add desired antibiotics. If necessary, prepare fresh complete IntestiCult™ Organoid Growth Medium (see Preparation section).
2. Thaw Matrigel® on ice; for each well to be passaged, 150 µL of Matrigel® will be required. Place DMEM/F-12 with 15 mM HEPES on ice.
3. Warm a tissue culture-treated 24-well plate in a 37°C incubator for at least 30 minutes.
4. Remove culture medium from each well to be passaged, without disturbing the dome of organoids in Matrigel®.
5. Add 1 mL of Gentle Cell Dissociation Reagent on top of the exposed dome in each well. Incubate at room temperature (15 - 25°C) for 1 minute.

6. Using a pre-wetted 1000 µL pipette tip, pipette the Gentle Cell Dissociation Reagent in the well up and down approximately 20 times to break up the dome and the organoids.
7. Using the same pipette tip, transfer the suspension to a 15 mL conical tube. Rinse the well with an additional 1 mL of Gentle Cell Dissociation Reagent and add to the same 15 mL conical tube.
8. Repeat steps 6 - 7 for each well to be passaged.
9. Incubate the 15 mL tubes containing the disrupted organoids on a rocking platform set at 20 rpm at room temperature (15 - 25°C) for 10 minutes.
10. Centrifuge the tubes at 290 x *g* for 5 minutes at 2 - 8°C. Gently pour off and discard the supernatant.
11. Using a pre-wetted 10 mL serological pipette, resuspend the pellets in 10 mL of cold (2 - 8°C) DMEM/F-12.
12. Centrifuge at 200 x *g* for 5 minutes at 2 - 8°C. Gently pipette off and discard the supernatant.

For subsequent steps in the passaging protocol, refer to section B steps 5 - 12.

For the cryopreservation of intestinal organoids, refer to the Technical Bulletin: Intestinal Epithelial Organoid Culture with IntestiCult™ Organoid Growth Medium (Mouse; Document #28223), available at www.stemcell.com or contact us to request a copy.

Related Products

For related products, including cytokines, supplements, dissociation reagents, and cultureware, visit www.stemcell.com/INTworkflow or contact us at techsupport@stemcell.com.