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. Intestinal organoids, or “mini-guts”, provide a convenient in vitro organotypic culture system for studying the intestinal epithelium and associated stem cell dynamics. Intestinal organoids grown in IntestiCult™ feature a polarized epithelium that contains all of the known cell types of the adult intestinal epithelium. The organoids incorporate a functional lumen, as well as the crypt-villus structure that characterizes the in vivo intestine. 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, modelling 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++ (PBS)	37350
Gentle Cell Dissociation Reagent	07174
DMEM/F12 with 15 mM HEPES	36254
Corning® Matrigel® Matrix, Growth Factor Reduced (GFR)	Corning 356230
Costar® 24 Well Clear Not Treated Multiple Well Plates	Corning 3738
Falcon® 70µm Cell Strainer	Corning 352350

Preparation of Complete IntestiCult™ Organoid Growth Medium (Mouse)

Use sterile techniques to prepare complete IntestiCult™ Organoid Growth Medium (Basal Medium + Supplement 1 + Supplement 2). 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. Mix thoroughly.

   NOTE: If not used immediately, store complete IntestiCult™ Organoid Growth Medium 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 complete IntestiCult™ Organoid Growth Medium (e.g. 100 units/100 µg per mL penicillin/streptomycin or 50 µg/mL gentamicin).

Directions for Use

Please read the entire protocol before proceeding.

Use sterile techniques when performing the following protocols.

For detailed instructions, refer to the Technical Bulletin: Intestinal Epithelial Organoid Culture with IntestiCult™ Organoid Growth Medium (Mouse; Document #28223).

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 Matrigel® Matrix, GFR on ice. You will need 150 µL for each preparation (4 wells of a 24-well plate).
2. Pre-warm a non-tissue culture-treated 24-well plate by placing in a 37°C incubator for at least 30 minutes.
3. Sacrifice mouse according to your approved institutional guidelines. Harvest approximately 20 cm of small intestine and place it in a 100 mm dish containing 5 mL of cold (2 - 8°C) PBS.
4. 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.
5. Use small scissors to make a longitudinal incision along the entire length of the intestine. Splay open the intestinal segment and use a micropipette 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.
6. Add 15 mL 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.
7. 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.
8. 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 at room temperature (15 - 25°C).
9. Let the intestinal pieces settle by gravity for approximately 30 seconds and carefully remove the supernatant.
10. Resuspend the intestinal pieces in 10 mL of cold (2 - 8°C) PBS + 0.1% bovine serum albumin (BSA) and pipette up and down 3 times.
11. Let most of the intestinal pieces settle to the bottom. Remove the supernatant and pass it through a 70 µm filter into a 50 mL conical tube. Label the filtrate “Fraction 1” and place on ice.
12. Repeat steps 10 - 11 three times to generate Fractions 2 - 4.
13. Centrifuge the 4 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.
14. Resuspend each pellet in 10 mL of cold (2 - 8°C) PBS + 0.1% BSA.
15. Transfer each suspension to a fresh 15 mL conical tube labeled with the corresponding fraction number.
16. Centrifuge the fractions at 200 x *g* for 3 minutes at 2 - 8°C. Gently pour off and discard the supernatants. Pelleted intestinal crypts will remain in the tubes.

B. INTESTINAL ORGANOID CULTURE

1. Resuspend each intestinal crypt pellet (section A) in 10 mL of cold (2 - 8°C) DMEM/F12.
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 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 per mL).
4. From the selected fraction(s), aliquot the crypts into 3 x 15 mL labelled 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® Matrix (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). These 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 5 - 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 PBS to any unused wells.
11. Place the lid on the culture plate and incubate at 37°C and 5% CO2.
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 typically start to bud after 2 - 4 days in culture.

C. PASSAGING OF MOUSE INTESTINAL ORGANOIDS

Mouse organoid cultures should be passaged every 7 - 10 days with an average split ratio of 1:6.

1. Thaw Matrigel® Matrix on ice. You will need 150 µL for each well to be passaged.
2. Pre-warm a non-tissue culture-treated 24-well plate by placing it in a 37°C incubator for at least 30 minutes.
3. Remove culture medium from each well to be passaged, without disturbing the dome of organoids in Matrigel®.
4. Add 1 mL of Gentle Cell Dissociation Reagent at room temperature (15 - 25°C) on top of the exposed dome in each well. Incubate for 1 minute at room temperature (15 - 25°C).
5. Using a 1 mL 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.
6. 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.
7. Repeat steps 5 - 6 for each well to be passaged.
8. Incubate the 15 mL tubes containing the disrupted organoids on a rocking platform at 20 rpm for 10 minutes at room temperature (15 - 25°C).
9. Centrifuge the tubes at 290 x *g* for 5 minutes at 2 - 8°C. Gently pour off and discard the supernatant.
10. Using a pre-wetted 10 mL serological pipette, resuspend the pellets in 10 mL of cold (2 - 8°C) DMEM/F12.
11. Centrifuge at 290 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.