IntestiCult™ OGM Human Basal Medium

Cell culture medium for establishment and maintenance of human colorectal cancer organoids

Catalog #100-0190 50 mL

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Product Description

IntestiCult™ Organoid Growth Medium (OGM) Human Basal Medium provides culture conditions optimal for Wnt-independent colorectal tumor organoid growth and expansion while maintaining tumor heterogeneity. The Wnt-free formulation ensures that Wnt-dependent normal, healthy intestinal cells do not contaminate the tumoroid culture. Tumor organoids generated using IntestiCult™ OGM Human Basal Medium are suitable for a variety of applications, including modeling tumor development and screening molecules for efficacy and toxicity.

For growing normal, Wnt-dependent organoids, use STEMCELL's IntestiCult™ Organoid Growth Medium (Human; Catalog #06010) or IntestiCult™-SF Organoid Growth Medium (Human; Catalog #100-0340). For differentiating human intestinal organoids, use IntestiCult™ Organoid Differentiation Medium (Human; Catalog #100-0214).

Should you intend to use this product for commercial purposes, please contact HUB at www.huborganoids.nl for a commercial use license or for clarification in relation to HUB licensing.

Properties

Storage: Store at -20°C.

NOTE: This product may be shipped with dry ice or ice packs and may be received thawed. Store components at -20°C upon receipt, or aliquot and store at -20°C. Do not exceed the component expiry date as indicated on the label.

Shelf Life: Stable until expiry date (EXP) 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.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
1.7 mL Microcentrifuge tubes	e.g. 38089
15 mL Conical tubes	e.g. 38009
25% Bovine serum albumin (BSA) in phosphate-buffered saline (PBS)	---
70 µm Reversible Strainer, Small	27216
Antibiotics (e.g. gentamicin or penicillin/streptomycin)	---
Corning® Matrigel® Matrix, Growth Factor Reduced (GFR), Phenol Red-Free	Corning 356231
Costar® 24 Well Flat-Bottom Plate, Tissue Culture-Treated	38017
D-PBS (Without Ca++ and Mg++)	37350
DMEM/F-12 with 15 mM HEPES	36254
Gentle Cell Dissociation Reagent	100-0485
Y-27632 (Dihydrochloride)	72302

Preparation of Reagents

A. INTESTICULT™ CANCER MEDIUM

Use sterile technique to prepare IntestiCult™ Cancer Medium (IntestiCult™ OGM Human Basal Medium + DMEM/F-12 with 15 mM HEPES). The following is for preparing 100 mL of IntestiCult™ Cancer Medium. If preparing other volumes, adjust accordingly.

1. Thaw IntestiCult™ OGM Human Basal Medium 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. Do not exceed the component expiry date as indicated on the label. After thawing aliquots, use immediately. Do not re-freeze.
2. Add 50 mL DMEM/F-12 with 15 mM HEPES to 50 mL IntestiCult™ OGM Human Basal Medium. Mix thoroughly.

   NOTE: If IntestiCult™ Cancer Medium is not used immediately, store 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 technique 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 technique when performing the following protocols.

A. ISOLATION OF HUMAN COLONIC CRYPTS FROM TUMOR BIOPSIES

1. Thaw 100 µL of Matrigel® on ice.

   NOTE: This is sufficient Matrigel® for plating up to 4 x 50 µL dome cultures. Depending on the crypt count (step 13), 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, wash the tissue sample with 10 mL of ice-cold D-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. Using a 1 mL pipettor, transfer the tissue and remaining supernatant to a 1.7 mL microcentrifuge tube.
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 D-PBS and add the rinse to the tissue fragments.
8. Allow the tissue fragments to settle by gravity (~5 seconds), then aspirate the supernatant.
9. Add 10 mL of Gentle Cell Dissociation Reagent (GCDR). Incubate at 37°C on a rocking platform set at medium speed (~40 RPM) for 60 minutes.
10. Centrifuge at 290 x *g* for 5 minutes. Aspirate the supernatant.

NOTE: For the remainder of section A, 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 to the tube with the fragments. 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.

    Using a 1 mL pipettor, pass the contents of the tube through a 70 µm 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.

13. Determine the total number of crypts in the sample as follows:
    a. Place 3 x 10 µL aliquots of the sample on an appropriate counting surface (e.g. a glass slide or 1 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
    Total number of crypts in 2 mL sample: 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.
14. 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 13, 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).

15. Remove the 24-well plate from the 37°C incubator. Pre-wet a 200 µL pipette tip with DMEM + 1% BSA.
16. Add 100 µL of Matrigel® to the sample tube. Pipette up and down 10 times to thoroughly resuspend the pellet. Avoid introducing bubbles.
17. 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.
18. Repeat step 17 until all of the Matrigel®-crypt suspension is dispensed.
19. 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.
20. Prepare 3 mL of complete IntestiCult™ Cancer 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 (Dihydrochloride) (10 µM final concentration). Mix thoroughly.

    NOTE: Each culture dome requires 750 µL of medium; 3 mL of medium is sufficient for 4 culture domes. If preparing a different number of culture domes, adjust volume of medium accordingly.
21. Add 750 µL of complete IntestiCult™ Cancer Medium (+ 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.

    NOTE: Y-27632 (Dihydrochloride) should be maintained in the medium for at least 2 days following crypt isolation to improve cell survival and organoid formation.
22. Add sterile D-PBS to unused wells.
23. Place the lid on the culture plate and incubate at 37°C and 5% CO_2.
24. Every 2 days, perform a full-medium change with complete IntestiCult™ Cancer Medium (Y-27632 [Dihydrochloride] is not required).
25. Proceed to section B for passaging.

B. 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 complete IntestiCult™ Cancer Medium 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 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 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 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 A, steps 13 - 25.

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.