IntestiCult™ Organoid Differentiation Medium (Human)

For the differentiation of intestinal organoids, or the establishment and maintenance of organoid-derived intestinal monolayers and air-liquid interface cultures

Catalog #100-0214 1 Kit

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 Differentiation Medium (Human) is a complete culture medium that supports the further differentiation of intestinal organoids in three dimensions (3D), or in 2D as monolayers or air-liquid interface (ALI) cultures. The starting cultures can be either intestinal organoids derived from human intestinal crypts, or passaged organoids that have been cultured with IntestiCult™ Organoid Growth Medium (Human; Catalog #06010).

Intestinal cultures generated using IntestiCult™ Organoid Differentiation Medium (Human) contain physiologically relevant proportions of differentiated and stem cell populations, recapitulating the diversity of the crypt-villus axis. When compared to conventional cell lines, intestinal monolayers exhibit greater barrier integrity, express higher levels of key differentiation markers, and have a morphology that is more representative of the in vivo intestine.

Applications of intestinal organoid cultures include studying the development and function of the intestinal epithelium, modeling intestinal diseases, compound screening, and regenerative therapy approaches. Intestinal monolayer and ALI cultuers are particularly amenable for permeability assays and studies of infectious dieases due to easy access to the apical surface. This kit requires IntestiCult™ Organoid Growth Medium (Human; Catalog #06010) for the initiation and expansion of intestinal organoids prior to differentiation.

Product Information

The following components are sold as a complete kit (Catalog #100-0214) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
IntestiCult™ ODM Human Basal Medium	100-0212	50 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
Organoid Supplement	100-0191	50 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
IntestiCult™ Organoid Growth Medium (Human)	06010
Dimethyl sulfoxide (DMSO)	e.g. Millipore Sigma D2650
3D Organoid Differentiation	
DAPT	72082
Monolayer Culture	
Corning® Matrigel® Matrix, Growth Factor Reduced (GFR), Phenol Red-Free	Corning 356231
Costar® 6.5 mm or 12 mm Transwell® inserts*	38023/38024
D-PBS (Without Ca++ and Mg++)	37350
Y-27632	72302
DMEM/F-12 with 15 mM HEPES	36254
Gentle Cell Dissociation Reagent	07174
Trypsin-EDTA (0.05%)	07910
Conical tubes, 15 mL	e.g. 38009

*Alternatively, standard tissue culture-treated plates may be used. However, if ALI culture is desired to increase differentiation of the epithelial layer (Directions for Use section II[C]), Transwell® inserts must be used for monolayer culture.

Preparation of Reagents and Materials

IntestiCult™ Organoid Differentiation Medium

Use sterile technique to prepare IntestiCult™ Organoid Differentiation Medium (IntestiCult™ ODM Human Basal Medium + Organoid Supplement + DAPT). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Prepare a 5 mM stock solution of DAPT in DMSO. Store at -20°C until ready to use.
2. Thaw Basal Medium and Organoid Supplement at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.
   NOTE: If not used immediately, aliquot and store at -20°C for up to 3 months. After thawing the aliquots, use immediately. Do not re-freeze.
3. Add 50 mL of Organoid Supplement to 50 mL of Basal Medium. Mix thoroughly.
4. Add 100 µL of 5 mM DAPT (final concentration 5 µM). Mix thoroughly.
   NOTE: 5 µM is the recommended concentration, but can be titrated up or down if desired.
   NOTE: If not used immediately, store at 2 - 8°C for up to 1 week.
5. Add desired antibiotics immediately before use (e.g. 50 µg/mL gentamicin or 100 units [100 µg/mL] penicillin/streptomycin).

IntestiCult™ Monolayer Growth Medium

Use sterile technique to prepare IntestiCult™ Monolayer Growth Medium (IntestiCult™ ODM Human Basal Medium + Organoid Supplement + Y-27632). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Prepare a 10 mM stock solution of Y-27632 in DMSO. Store at -20°C until ready to use.
2. Thaw Basal Medium and Organoid Supplement at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.
   NOTE: If not used immediately, aliquot and store at -20°C for up to 3 months. After thawing the aliquots, use immediately. Do not re-freeze.
3. Add 50 mL of Organoid Supplement to 50 mL of Basal Medium. Mix thoroughly.
4. Add 100 µL of 10 mM Y-27632 (final concentration 10 µM). Mix thoroughly.
   NOTE: If not used immediately, store at 2 - 8°C for up to 1 week.
5. Add desired antibiotics immediately before use (e.g. 50 µg/mL gentamicin or 100 units [100 µg/mL] penicillin/streptomycin).

Coating Cultureware with Corning® Matrigel®

For monolayer organoid culture (section II), cultureware must be coated with Corning® Matrigel®, as described below. For optimal results, Costar® 6.5 mm or 12 mm Transwell® inserts (Catalog #38023/38024) are recommended; however, standard tissue culture-treated plates may be used. If ALI culture is desired to increase differentiation of the epithelial layer (section II[C]), Transwell® inserts must be used for monolayer culture.

NOTE: Defined matrices such as Collagen I (e.g. Catalog #04902), Collagen IV, or Vitronectin XF™ (Catalog #07180) can be used instead of Matrigel®, but protocols may require further optimization.

1. Thaw one aliquot of Corning® Matrigel® on ice.
2. Dispense an appropriate amount of cold (2 - 8°C) D-PBS into a 15 mL conical tube and place on ice. Refer to Table 1 for recommended volumes.

Table 1. Recommended Volumes of Diluted Matrigel® for Various Cultureware

CULTUREWARE	VOLUME OF DILUTED MATRIGEL® PER WELL
6.5 mm Transwell® insert	100 µL (top)
12 mm Transwell® insert	250 µL (top)
6-well plate	1000 µL
24-well plate	250 µL
96-well plate	100 µL

3. Add thawed Matrigel® to the cold D-PBS at a ratio of 1 µL Matrigel® to 49 µL D-PBS. Mix thoroughly.
4. Immediately coat cultureware with diluted Matrigel®. Swirl the cultureware to spread the solution evenly across the surface.
5. Incubate at 37°C for at least 1 hour before use. Do not let the Matrigel® solution evaporate.
   NOTE: If not used immediately, seal the cultureware with Parafilm® to prevent evaporation; store at 2 - 8°C for up to 1 week after coating. Allow stored coated cultureware to warm to room temperature (15 - 25°C) for 30 minutes before proceeding to the next step.
6. Gently tilt the cultureware to one side and allow the excess Matrigel® solution to collect at the edge. Remove the excess Matrigel® solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched.

Directions for Use

Refer to section I for 3D organoid differentiation or section II for monolayer culture.

I. Intestinal Organoid Differentiation

Please read the entire protocol before proceeding.

Use sterile technique when performing the following protocols:

A. Initiation of Human Colonic Organoid Cultures

B. 3D Organoid Differentiation

A. INITIATION OF HUMAN COLONIC ORGANOID CULTURES

1. Initiate human colonic organoid cultures from biopsies or passaged cultures (< 15 passages) as described in the Product Information Sheet (PIS) for IntestiCult™ Organoid Growth Medium (Human).
2. After passaging and replating as described in the IntestiCult™ Organoid Growth Medium PIS, incubate organoid cultures at 37°C for 4 - 6 days in IntestiCult™ Organoid Growth Medium, performing a full-medium change every 2 - 3 days. If using a 24-well plate, aim for a density of 100 - 200 organoids per well. Organoids will appear as cystic structures with a diameter of 100 - 200 µm.

   NOTE: Prior to this point, organoids are too small to collect enough material for downstream analysis (ICC, qPRC, and functional assays). Intestinal organoid cultures exhibit a wide range of donor-to-donor variability; this is reflected in growth rates and morphology. Organoids do not have to be cystic to initiate differentiation, but they should be at least 100 µm in diameter. Typically, cultures at earlier passage numbers have more budding, and cultures at later passages are more cystic.
3. Proceed to section B for differentiation.

B. 3D ORGANOID DIFFERENTIATION

Once an organoid line has been established (section A), proceed with differentiation as described below.

1. Prepare IntestiCult™ Organoid Differentiation Medium (see Preparation section).
2. Remove organoid culture from the incubator (prepared in section A). With the culture plate tilted slightly (20 degrees off the bench), slide a 1 mL pipette tip along the wall of the well and pipette off medium from organoid domes. Be careful not to disrupt the organoid domes.
3. Add 750 µL of IntestiCult™ Organoid Differentiation Medium along the side wall of each well. Be careful not to disturb the organoid domes. Incubate at 37°C for 5 - 7 days, performing a full-medium change every 2 days.

   NOTE: As cultures grow in IntestiCult™ Organoid Differentiation Medium, the epithelium should thicken and darken, and budding structures should become more pronounced. If organoids begin to crumble or their membrane appears to become rough (rather than smooth), terminate differentiation.
4. Proceed with downstream analyses.

II. Monolayer Culture

Please read the entire protocol before proceeding.

Use sterile technique when performing the following protocols:

A. Dissociation of Human Intestinal Organoids

B. Plating Organoid Fragments for Monolayer Culture

C. Air-Liquid Interface (ALI) Differentiation (Optional)

D. Organoid Suspension Culture (Optional)

A. DISSOCIATION OF HUMAN INTESTINAL ORGANOIDS

For complete instructions for growing human intestinal organoids using IntestiCult™ Organoid Growth Medium (Human), refer to the corresponding Product Information Sheet. The following protocol is for dissociating 2 - 3 wells of human intestinal organoids grown in IntestiCult™ Organoid Growth Medium (Human) in a 24-well plate for 7 - 8 days in 50 µL 50% Matrigel® domes. If seeding monolayer cultures on a larger scale, we recommend using organoids grown in suspension culture to increase organoid density per well (see section D).

Intestinal organoids should be passaged no more than 15 times if being used for monolayer cultures; seeding efficiency may vary with organoid passage number and the number of days growth. This protocol assumes at least 100 - 150 organoids per well. Matrigel® domes may contain fewer but larger organoids, or a larger number of smaller organoids, but will yield similar results. The number of organoids required may vary with donor and culture quality and may need to be further optimized.

1. Refer to Table 2 for the recommended number of wells of intestinal organoids to harvest for various cultureware.

   NOTE: If ALI culture is desired to increase differentiation of the epithelial layer (section C), Transwell® inserts must be used for monolayer culture.

Table 2. Recommended Number of Wells of Intestinal Organoids to Harvest

MONOLAYER CULTUREWARE	NUMBER OF WELLS OF INTESTINAL ORGANOIDS TO HARVEST (per well to be seeded)
6.5 mm Transwell® insert	2 - 3 wells
12 mm Transwell® insert	3 - 4 wells
6-well plate	6 - 8 wells
24-well plate	3 - 4 wells
96-well plate	1 - 2 wells

2. Aspirate all media from the organoid cultures without disturbing the organoids within the Matrigel® dome.
3. Add 1 mL of Gentle Cell Dissociation Reagent to each well of organoids to be harvested.
4. Incubate at room temperature (15 - 25°C) for 1 minute.
5. Using a 1 mL pipettor, vigorously pipette up and down to disrupt the Matrigel® dome and resuspend the organoids.
6. Pool the harvested wells in a 15 mL conical tube. Incubate at room temperature for 10 minutes with gentle agitation or rocking.
7. Centrifuge at 200 x *g* for 5 minutes at 2 - 8°C.
8. Remove and discard the supernatant. Add 5 mL ice-cold DMEM/F-12 with 15 mM HEPES to resuspend organoids. Centrifuge at 200 x *g* for 5 minutes at 2 - 8°C.
9. Aspirate supernatant, removing as much as possible, being careful not to disturb the pellet. Add 1 mL of warm (37°C) Trypsin-EDTA (0.05%) to resuspend organoids.

   NOTE: If pooling larger numbers of organoid wells, increase the volume of Trypsin-EDTA to ensure efficient dissociation of the organoids.
10. Using a 1 mL pipettor, pipette up and down to mix thoroughly. Incubate at 37°C for 5 - 10 minutes.
11. Mix thoroughly by vigorous pipetting or vortexing to disrupt the organoids as much as possible. Use a microscope to check the organoids for sufficient disruption. Organoids should be dissociated into either individual cells or small fragments. If many large fragments or whole organoids remain, repeat pipetting/vortexing until fragments are sufficiently disrupted.

    NOTE: Perform the remaining steps as quickly as possible, as cells will start to clump together.
12. Add an equal volume of DMEM/F-12 (e.g. 1 mL DMEM/F-12 per mL Trypsin-EDTA) and pipette up and down to mix thoroughly. Centrifuge fragments at 200 x *g* for 5 minutes at 2 - 8°C.

    NOTE: DMEM/F-12 supplemented with 5 - 10% FBS can also be used to neutralize Trypsin-EDTA if needed. Carefully remove and discard the supernatant.

    NOTE: If a buoyant mucus layer is present, steps 11 and 12 may need to be repeated to properly pellet the organoid fragments.
13. Proceed to section B for plating.

B. PLATING ORGANOID FRAGMENTS FOR MONOLAYER CULTURE

1. Coat cultureware with Corning® Matrigel® and prepare IntestiCult™ Monolayer Growth Medium (see Preparation section).
2. Add IntestiCult™ Monolayer Growth Medium (prepared in section A) to organoid fragments and mix gently. Refer to Table 3 for the volume required for various cultureware.

Table 3. Recommended Resuspension Volume of IntestiCult™ Monolayer Growth Medium for Various Cultureware

CULTUREWARE	VOLUME OF INTESTICULT™ MONOLAYER GROWTH MEDIUM PER WELL
6.5 mm Transwell® insert	100 µL (top), 500 µL (bottom)
12 mm Transwell® insert	500 µL (top), 1.5 mL (bottom)
6-well plate	1.5 mL
24-well plate	500 µL
96-well plate	100 µL

3. Slowly and gently add the fragment suspension to each Matrigel®-coated well. Incubate at 37°C and 5% CO_2. Monitor growth daily. Perform a full-medium change every 2 - 3 days.

   NOTE: Monolayers will usually reach 100% confluency within 2 - 3 days; however, if attachment is poor, this may take longer. Cells will remain viable and the monolayer will remain confluent for at least 3 weeks, with continued full-medium changes every 2 - 3 days.
4. For air-liquid interface (ALI) culture, proceed to section C.

C. AIR-LIQUID INTERFACE (ALI) DIFFERENTIATION (OPTIONAL)

The following instructions are for using an ALI culture to increase differentiation of the epithelial layer. This is an optional step that is not necessary for all applications. The result will be a significant increase in the number of secretory and other differentiated cell types at the expense of stem cells and transit-amplifying cells.

Organoid-derived human intestinal epithelial cell monolayer cultures should be established in a Transwell® insert (sections A & B). When the monolayer has been **100% confluent for at least 4 days**, proceed with the protocol below.

1. Remove medium from the top and bottom wells. Add fresh IntestiCult™ Monolayer Growth Medium to the bottom well; leave the top well empty. Incubate at 37°C and 5% CO_2.
2. Every 2 - 3 days, perform a full-medium change in the bottom well using fresh IntestiCult™ Monolayer Growth Medium. Allow the monolayer to differentiate for at least 1 week. The ALI culture can be maintained for 2 weeks or longer, with full-medium changes every 2 - 3 days.

   NOTE: The upper well can be washed with 100 µL D-PBS to rinse away excess mucus; however, this is not necessary and the presence of mucus may be desirable to better represent the physiology of the intestinal epithelium.

D. ORGANOID SUSPENSION CULTURE (OPTIONAL)

The protocol below is an alternative to the protocol in the Product Information Sheet (PIS) for IntestiCult™ Organoid Growth Medium (Human), in which organoids are suspended in Matrigel® domes. This protocol describes how to passage organoids from Matrigel® domes into a suspension culture, 7 days prior to establishing monolayer cultures. This alternative method for organoid growth will facilitate a higher number of organoids per well, reducing the amount of work needed when establishing larger numbers of organoid monolayers.

1. Start with organoid cultures grown in 50 µL 50% Matrigel® domes in a 24-well plate as described in the IntestiCult™ Organoid Growth Medium (Human) PIS. Each well of a 6-well suspension plate will require 4 - 6 wells of organoids (600 - 1000 organoids).
2. Prepare IntestiCult™ Organoid Growth Medium (Human) and warm to room temperature (15 - 25°C).

   NOTE: For each well of a 6-well suspension plate, 3 mL of medium will be required.
3. Add 3 mL of IntestiCult™ Organoid Growth Medium (Human) per well of a 6-well Ultra-Low Adherent Plate for Suspension Culture (Catalog #38071). Place in a 37°C incubator for at least 1 hour.
4. Thaw Corning® Matrigel® on ice; for each well of a 6-well plate, 500 µL of Matrigel® will be required. Place DMEM/F-12 with 15 mM HEPES 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) Gentle Cell Dissociation Reagent (GCDR) on top of the exposed dome in each well. Incubate at room temperature for 1 minute.
7. Pre-wet a 1 mL pipette tip with GCDR; use this pipette tip to thoroughly scrape the Matrigel® dome free of the bottom of the well. Pipette the GCDR in the well up and down 2 - 3 times to break up the dome and the organoids; avoid touching the bottom of the well with the pipette tip. Ensure all pieces of Matrigel® have been rinsed free of the plate.
8. Using the same pipette tip, transfer the organoid mixture to a 15 mL conical tube.
9. Add 1 mL 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 5 mL of cold DMEM/F-12 to each tube. Using a pre-wetted 1 mL pipette tip, vigorously pipette up and down 15 times to resuspend and fragment organoids.

    NOTE: Avoid touching the side/bottom of the tube with the pipette tip.
14. Centrifuge at 200 x *g* for 5 minutes. Aspirate as much supernatant as possible.
15. Resuspend the organoid fragments in each tube in 500 µL cold Matrigel®. Mix thoroughly by gently pipetting up and down 10 - 15 times (try to avoid introducing air bubbles). Always keep the Matrigel® suspension on ice.

    NOTE: Add 10 µM Y-27632 to increase organoid yield.

16. Remove the 6-well suspension plate from the incubator (prepared in step 3). Slowly pipette the organoid-Matrigel® suspension into the center of the medium in one well. The Matrigel® should immediately solidify and remain suspended in the medium. Repeat for each tube of organoid-Matrigel® suspension.
17. Incubate at 37°C and 5% CO_2. Cultures can be maintained for up to 7 days. Perform a half-medium change every 2 - 3 days, as follows:
    a. Tilt the plate and use a pipette tip to gently push the Matrigel® suspension away from a corner of the well.
    b. Use the pipette to gently remove 1.5 mL of medium (do not aspirate). Be careful not to remove any of the Matrigel® suspension.
    c. Add 1.5 mL fresh IntestiCult™ Organoid Growth Medium (Human).

    NOTE: Each well of a 6-well suspension plate should yield at least an equivalent number of organoids as 18 wells of a 24-well plate with dome cultures (1800 - 2400 organoids).
18. To establish a monolayer culture, transfer the organoid suspension from one well of the 6-well suspension plate to a 15 mL conical tube. Centrifuge at 200 x *g* for 5 minutes. Aspirate as much supernatant as possible.
19. Add 5 mL GCDR to the organoid pellet and mix gently to resuspend. Incubate at room temperature for 10 minutes with gentle agitation or rocking.
20. Proceed to section II-A, step 7 to dissociate organoids for monolayer culture.