HepatiCult™ Organoid Growth Medium (Mouse)

Cell culture medium for establishment and maintenance of mouse hepatic progenitor organoids

Catalog #06030 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

HepatiCult™ Organoid Growth Medium (Mouse) is a serum-free, defined cell culture medium for establishment and maintenance of mouse hepatic progenitor organoids. These organoids, or "mini-livers", provide an in vitro organotypic culture system for studying hepatic stem and progenitor cells. Organoids grown in HepatiCult™ feature an epithelium expressing genes marking hepatic stem and progenitor cells (PROM1, AXIN2, SOX9 and CD44), ducts (KRT19 and HNF1b), and hepatocytes (HNF4a, AFP). Hepatic organoids can be passaged every 4 - 7 days, can be cryopreserved, and are primed for downstream differentiation.

HepatiCult™ supports mouse hepatic organoid culture either embedded in Corning® Matrigel® domes or in a dilute Matrigel® suspension. Organoid culture enables convenient in vitro characterization of the hepatic epithelium in a physiologically relevant system and reduces the need for animal use.

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.

Product Information

The following components are sold as a kit (Catalog #06030) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
HepatiCult™ OGM Mouse Basal Medium	06031	95 mL	Store at 2 - 8°C.	Stable for 2 years from date of manufacture (MFG) on label.
HepatiCult™ OGM Mouse Supplement*	06032	5 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.

*This component 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 #
DMEM/F-12 with 15 mM HEPES	36254
Advanced DMEM/F-12	Thermo Fisher 12634010
Anti-Adherence Rinsing Solution	07010
Collagenase Type IV (1 mg/mL)	07909
Dispase (1 U/mL)	07923
100 mm Culture Dish, Non-Treated	38045
Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix, Phenol Red-Free, LDEV-Free*	Corning 356231*
D-PBS (Without Ca++ and Mg++)	37350
Antibiotics (e.g. gentamicin)	---
37 µm Reversible Strainer, Large	27250
70 µm Reversible Strainer, Large	27260
Falcon® Conical Tubes	38009 (15 mL) and 38010 (50 mL)
Falcon® Serological Pipettes	38004 (10 mL) and 38005 (25 mL)
Costar® 24-Well Flat-Bottom Plate, Tissue Culture-Treated	38017
Costar® 6-Well Flat-Bottom Plate, Tissue Culture-Treated	38015

*We recommend using Corning® Matrigel® lots containing ≥ 8 mg/mL protein. Lower protein concentrations may affect organoid growth.

Preparation of Reagents and Materials

HepatiCult™ Organoid Growth Medium (Mouse)

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

1. Thaw the Supplement at 2 - 8°C overnight. Mix thoroughly.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquots, use immediately. Do not re-freeze.
2. Add 5 mL of Supplement to 95 mL of Basal Medium.
3. Add antibiotics (e.g. 50 µg/mL gentamicin). Mix thoroughly. Warm to room temperature (15 - 25°C) before use.

   NOTE: If not used immediately, store HepatiCult™ Organoid Growth Medium at 2 - 8°C for up to 1 month.

Tissue Dissociation Cocktail

1. Use sterile technique to prepare 60 mL of Tissue Dissociation Cocktail (sufficient for one mouse liver) by combining the following:
   - 7.5 mL Collagenase Type IV
   - 7.5 mL Dispase
   - 45 mL DMEM/F-12 with 15 mM HEPES

   Mix thoroughly.

   NOTE: If not used immediately, store at 2 - 8°C for up to 1 month.
2. Warm to room temperature (15 - 25°C) before use.

Pre-Wetting Conical Tubes and Serological Pipettes

Conical tubes and serological pipettes that come in contact with hepatic ducts should be pre-wetted, as hepatic ducts frequently adhere to their surfaces, significantly reducing organoid yield. Pre-wet tubes and pipettes on the day of the experiment.

For each liver being processed, pre-wet **4 x 15 mL conical tubes** and **1 x 50 mL conical tube** as follows:

1. Add 5 mL of Anti-Adherence Rinsing Solution to a 15 mL conical tube. Swirl to coat the tube.
2. Transfer the entire volume to a second 15 mL conical tube. Repeat until all 15 mL conical tubes have been coated. Discard the used Rinsing Solution.
3. Aspirate any remaining Rinsing Solution from the coated tubes.
4. Repeat steps 1 - 2 using Advanced DMEM/F-12 instead of Rinsing Solution. Aspirate any remaining Advanced DMEM/F-12 from coated 15 mL conical tubes.
5. To pre-wet 1 x 50 mL conical tube, use 30 mL Anti-Adherence Rinsing Solution and 30 mL Advanced DMEM/F-12 and follow steps 1 - 4.
6. Cap all coated tubes tightly and store at room temperature (15 - 25°C) until required.

Pre-wet **1 x 10 mL serological pipette** as follows:

1. Add 10 mL of Anti-Adherence Rinsing Solution to a 15 mL conical tube. Add 10 mL of Advanced DMEM/F-12 to a second 15 mL conical tube. Place aliquots at room temperature.
2. Immediately before use in section A step 20, coat serological pipette with aliquoted Anti-Adherence Rinsing Solution, followed by Advanced DMEM/F-12.

Directions for Use

Please read the entire protocol before proceeding. Use sterile technique when performing the following protocols:

A. Organoid Initiation

B. Passaging Mouse Hepatic Progenitor Organoids

A. ORGANOID INITIATION

The following protocol is for initiating hepatic progenitor organoids using hepatic duct fragments from one mouse, and culturing in Matrigel® domes. For protocols using single cells and for culture in a dilute Matrigel® suspension, refer to the Technical Bulletin: Hepatic Progenitor Organoid Culture: Supplementary Protocols (Document #DX27087), available at www.stemcell.com or contact us to request a copy.

SET-UP

1. Place a 24-well tissue culture-treated plate in a 37°C incubator for at least 1 hour. Place a box of sterile 200 µL tips at 2 - 8°C.

2. Thaw 150 - 200 µL of Corning® Matrigel® on ice.

   NOTE: Keep Matrigel® on ice when thawing and handling to prevent it from solidifying.
3. Prepare HepatiCult™ Organoid Growth Medium and Tissue Dissociation Cocktail. Warm to room temperature (15 - 25°C).
4. Add 30 mL of DMEM/F-12 with 15 mM HEPES to each of 2 x 100 mm dishes and place on ice. Place 2 x 50 mL conical tubes on ice.

ISOLATION OF HEPATIC DUCT FRAGMENTS

5. Sacrifice mouse according to approved institutional guidelines. Keep mouse on ice until dissection.
6. Within 2 hours of sacrifice, harvest the mouse liver and transfer it to a dish containing cold DMEM/F-12 (prepared in step 4).
7. Swirl liver in dish to wash. Use forceps to transfer liver to the second dish containing cold DMEM/F-12.
8. With liver submerged in DMEM/F-12, cut up liver into small (~3 - 5 mm) pieces.
9. Using a 25 mL serological pipette, transfer liver pieces and DMEM/F-12 into a 50 mL tube on ice (prepared in step 4).
10. Let liver pieces settle by gravity on ice for 2 minutes. Aspirate and discard supernatant.
11. Add 10 mL of room temperature Tissue Dissociation Cocktail (see Preparation section) to liver pieces. Incubate tube in a 37°C water bath for 20 minutes.
12. Remove tube from water bath. Using a 10 mL serological pipette, vigorously pipette liver pieces up and down 7 times with medium-high force.
13. Let liver pieces settle by gravity for 1 minute.
14. Using a 10 mL serological pipette, remove and discard the supernatant (including digested cells suspended in it).

    NOTE: The digestion supernatant is only discarded in this first digestion cycle. In the remaining digestion cycles, retain the supernatant as indicated.
15. Repeat steps 11 - 13 (digestion cycle #2). Using a 10 mL serological pipette, collect supernatant and add to a new cooled 50 mL tube (prepared in step 4). Keep on ice.
16. Repeat steps 11 - 13, collecting supernatant into the 50 mL tube on ice (from step 15) after each digestion. Continue repeating these steps until liver pieces have been dissociated into hepatic ducts and no liver pieces remain. This usually requires up to 6 x 20-minute digestion cycles. The pooled supernatant volume from digestion cycles #2 - 6 will be ~50 mL.
17. Attach a 70 µm cell strainer to a new 50 mL conical tube. Using a 25 mL serological pipette, pass the pooled supernatant (from step 16) through the strainer. Discard the strainer.
18. Attach a 37 µm Reversible Strainer to a new 50 mL conical tube. Using a 25 mL serological pipette, pass the flow-through from step 17 through the strainer. Discard the new flow-through.
19. Reverse the strainer onto a pre-wetted 50 mL conical tube (see Preparation section). Using a 10 mL serological pipette, carefully add 10 - 12 mL of cold (2 - 8°C) Advanced DMEM/F-12 to the reversed strainer to wash hepatic ducts into the pre-wetted tube.

    NOTE: Ensure the tip of the serological pipette touches the surface of the strainer while the entire bottom surface is thoroughly rinsed. If fragments still remain on the surface, use a serological pipette to gently scrape them off and transfer to the tube.
20. Using a pre-wetted 10 mL serological pipette (see Preparation section), pipette the hepatic ducts (collected in step 19) up and down 3 - 5 times to create an even suspension. Immediately transfer equal volumes into each of 4 x 15 mL pre-wetted conical tubes (see Preparation section).
21. Centrifuge the 4 tubes at 290 x *g* for 5 minutes. Aspirate as much of the supernatant as possible without disturbing the pelleted ducts, leaving ~5 - 10 µL in each tube (the pellet is often not visible). Place tubes on ice.

CULTURE IN MATRIGEL® DOMES

22. Remove the 24-well plate from the incubator and 200 µL pipette tips from the fridge and place in a biosafety cabinet.
23. Process one tube/pellet at a time, as described below. Work quickly to ensure the Matrigel® does not solidify.

    NOTE: The 8 wells in the center of a 24-well plate are the most suitable for domes since their surfaces are the most even. Wells at the edges of the plate are often slightly slanted, resulting in domes touching the wall of the well and flattening out.

    a. Using a pipettor with a cooled 200 µL pipette tip, add 30 µL of thawed Matrigel® on top of the pellet. Without generating bubbles, gently mix the duct-Matrigel® suspension by pipetting up and down 5 - 8 times, going to only the first stop of the pipettor.

    b. Set the pipettor volume to 50 µL. Add the entire suspension to the center of 1 well of the 24-well plate to form a dome. While dispensing, gradually move the pipette tip upwards so that the ducts are evenly distributed throughout the dome. Dispense only to the first stop of the pipettor to avoid generating bubbles on top of the dome.
24. Repeat step 23 for the remaining pellets.
25. Place the lid on the culture plate. Carefully place the plate in an incubator at 37°C and 5% CO_2 for 10 minutes to let domes solidify.
26. Remove the plate from the incubator and place in the biosafety cabinet.

27. Without disturbing the domes, carefully add 750 µL of room temperature (15 - 25°C) HepatiCult™ Organoid Growth Medium against the side of each well containing a dome. Do not pipette directly onto the domes.
28. Add sterile PBS to any unused wells. Place the lid on the culture plate.
29. Capture one 2X image per dome using a brightfield microscope (Day 0 images). Incubate the plate at 37°C and 5% CO_2.

    NOTE: To monitor organoid growth, take photos of the same field of view every 2 - 3 days until they are passaged.
30. Perform a full-medium change every 2 - 3 days for up to 1 week by carefully aspirating the medium and adding 750 µL of fresh HepatiCult™ Organoid Growth Medium at room temperature.

    NOTE: If Matrigel® domes are loose, remove 250 µL of medium from the well, then add 500 µL of fresh medium.

    NOTE: To avoid weekend medium changes, perform medium changes on Mondays, Wednesdays, and Fridays.
31. Monitor organoids daily. They should be passaged before the lumen turns dark and collapses. During early passages, this usually occurs at Day 4 - 6, and during later passages at Day 6 - 7. Proceed to section B when organoids are ready for passaging.

B. PASSAGING MOUSE HEPATIC PROGENITOR ORGANOIDS

The following protocol is for passaging hepatic progenitor organoids via fragment number followed by culture in Matrigel® domes. For single-cell passaging and for culture in a dilute Matrigel® suspension, refer to the Technical Bulletin: Hepatic Progenitor Organoid Culture: Supplementary Protocols (Document #27087), available at www.stemcell.com, or contact us to request a copy.

1. Place a 24-well plate in a 37°C incubator for at least 1 hour. Place a box of sterile 200 µL pipette tips at 2 - 8°C.
2. Thaw Matrigel® on ice (~40 µL/well to be passaged). Place Advanced DMEM/F-12 on ice.
3. Prepare HepatiCult™ Organoid Growth Medium and bring to room temperature (15 - 25°C).
4. Check that the Matrigel® domes to be passaged are intact. If the domes are intact, proceed to step 5. If the domes are loose, add cold Advanced DMEM/F-12 to top up the total volume in the well to 1 mL and let sit for 1 minute; proceed to step 7.
5. Without touching the dome, aspirate and discard the medium in each well to be passaged.
6. Using a 1 mL pipettor, forcefully add 1 mL of cold Advanced DMEM/F-12 to the center of each dome and let sit for 1 minute.
7. Using a 1 mL pipette tip on the pipettor, vigorously pipette the total volume up and down 15 times, taking care not to generate bubbles.

    NOTE: This results in mechanical breakdown of organoids and Matrigel® into smaller fragments of 30 - 100 µm. Check fragment sizes using a light microscope; if most fragments are larger than 100 µm, triturate until they are ≤ 100 µm.
8. Combine the contents of all wells into one 15 mL conical tube.
9. Vortex the tube containing organoid fragments gently at medium speed for 5 seconds. Immediately transfer 3 x 10 µL of fragment suspension into an empty well of a 6-well plate to create 3 separate droplets. Place the remaining fragment suspension on ice.
10. Determine the average number of organoid fragments in suspension as follows:
    a. Using a light microscope, count the number of hepatic organoid fragments in each 10 µL droplet and determine the average number of fragments in 10 µL. Only count fragments that are 30 - 100 µm.

       NOTE: If the fragment density is too high to count, dilute the suspension using Advanced DMEM/F-12 and repeat step 9.
    b. Calculate the volume required to transfer 200 fragments/well to the next passage. For each new Matrigel® dome to be seeded, add this volume to a 15 mL conical tube containing 1 mL of Advanced DMEM/F-12.

    Example:

    3 x 10 µL fragment counts = *35, 40, 42 fragments*

    Average fragment count per 10 µL = *39 fragments*

    Volume from fragment suspension to transfer 200 fragments = *51 µL*
11. Centrifuge tubes containing 200 fragments each (prepared in step 10b) at 290 x *g* for 5 minutes. Aspirate as much of the supernatant as possible without disturbing the pellets, leaving ~5 - 10 µL in the tube (the pellet is often not visible). Place tubes on ice.
12. For subsequent steps in the passaging protocol, refer to section A steps 22 - 30.

    NOTE: Monitor hepatic progenitor organoids daily. Organoids seeded with 200 fragments typically require passaging every 4 - 7 days; after the initial passage, use a 1:10 to 1:30 split ratio in subsequent passages (fragment counting is not required).

Related Products

For related products, including specialized culture and storage media, dissociation reagents, antibodies, cytokines, and small molecules, visit www.stemcell.com, or contact us at techsupport@stemcell.com.