

# Mouse Intestinal Organoids

## Cryopreserved mouse intestinal epithelial organoids for establishment of organoid cultures

Catalog #70931

200 Organoids



Scientists Helping Scientists™ | [WWW.STEMCELL.COM](http://WWW.STEMCELL.COM)

TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713

[INFO@STEMCELL.COM](mailto:INFO@STEMCELL.COM) • [TECHSUPPORT@STEMCELL.COM](mailto:TECHSUPPORT@STEMCELL.COM)

FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

## Product Description

Cryopreserved Mouse Intestinal Organoids provide a convenient way to establish or standardize intestinal organoid cultures. Each vial contains 200 mouse intestinal organoids derived from the small intestine of C57BL/6 mice that were cultured in IntestiCult™ Organoid Growth Medium (Mouse; Catalog #06005) and cryopreserved in CryoStor® CS10 (Catalog #07930).

Using cryopreserved Mouse Intestinal Organoids enables establishment of intestinal organoid cultures without the need to isolate intestinal crypts from primary tissue, eliminating the need for access to fresh mouse tissue and making it easy to standardize experimental starting materials. The organoids can be passaged and expanded using IntestiCult™ Organoid Growth Medium (Mouse) and refrozen in CryoStor® CS10. Mouse intestinal organoid cultures can be used for research in a variety of fields, including epithelial cell biology, cancer, cystic fibrosis, microbiomics, intestinal immunology, and bacterial or viral pathogenesis.

Should you intend to use this product for commercial purposes, please contact HUB at [www.huborganoids.nl](http://www.huborganoids.nl) for a commercial use license or for clarification in relation to HUB licensing.

## Properties

**Storage:** Store at -135°C or colder.

**Shelf Life:** Stable for 5 years from date of manufacture (MFG) on label.

**Contains:**

- Frozen mouse intestinal organoid segments
- CryoStor® CS10

## Materials Required but Not Included

| PRODUCT NAME  | CATALOG #      |
|---|----------------|
| 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          |
| DMEM/F-12 with 15 mM HEPES  | 36254          |
| IntestiCult™ Organoid Growth Medium (Mouse)                             | 06005          |

## Directions for Use

The following instructions are for preparing one cryovial of organoids for 4 wells of a 24-well plate. Use sterile technique throughout the protocol.

NOTE: Pre-wet pipette tips with DMEM/F-12 with 15 mM HEPES + 1% BSA before manipulating organoids. This prevents tissue from sticking to the wall of the pipette tip.

### SETUP

1. Thaw 120 µL of Matrigel® on ice.
2. Prepare complete IntestiCult™ Organoid Growth Medium (refer to the Product Information Sheet for IntestiCult™ [Document #10000003509]). Warm to room temperature (15 - 25°C).

NOTE: For 4 wells of a 24-well plate, 3.1 mL of complete IntestiCult™ Organoid Growth Medium is required.

3. Warm a 24-well tissue culture-treated plate in a 37°C incubator for 30 minutes.
4. Prepare DMEM/F-12 with 15 mM HEPES + 1% BSA (DMEM + BSA) as follows: Add 2 mL of 25% BSA to 48 mL of DMEM/F-12 with 15 mM HEPES. Mix thoroughly. Store at room temperature for the duration of the protocol. Store the remaining DMEM + BSA at 2 - 8°C for up to 1 month.

- To a 15 mL conical tube, add 2 mL of DMEM + BSA (prepared in step 4).

NOTE: Transfer cells to this tube immediately after thawing (steps 6 - 8) to avoid a significant reduction in viability.

#### THAWING ORGANOIDS

- Place the cryovial of organoids in a 37°C water bath to thaw for 2 - 2.5 minutes. Thawing is complete when the freezing medium becomes liquid and the organoids are visible at the bottom of the tube. Perform steps 7 - 8 immediately after cells are thawed.

NOTE: Warming the frozen organoids for too long may affect the growth of the organoids in culture. Once thawed, do not re-freeze.

- Wipe the outside of the cryovial with 70% ethanol or isopropanol before opening.
- Using a 1 mL pipettor, add 1 mL of DMEM + BSA to the cryovial. Using the same pipette tip, mix the contents of the cryovial by pipetting up and down 4 times. Immediately transfer the contents of the cryovial to the tube prepared in step 5.
- Wash the entire surface area of the cryovial and inside of the lid with 2 x 1 mL of DMEM + BSA. Add the washes to the organoid suspension.
- Centrifuge the organoid suspension at 200 x g for 5 minutes. If there are bubbles on the surface, aspirate them before aspirating the remainder of the supernatant.

#### CULTURING ORGANOIDS IN MATRIGEL® DOMES

- Remove the plate from the incubator and place in the biosafety cabinet.
- Using a 200 µL pipettor, add 100 µL of complete IntestiCult™ Organoid Growth Medium to the organoid pellet. Using the same pipette tip, mix by pipetting up and down until organoids are resuspended, approximately 5 - 10 times.
- Using a 200 µL pipettor, add 100 µL of cold Matrigel® to the tube. Using a pre-wetted pipette tip, mix the suspension by pipetting up and down 5 - 10 times.
- Using a pipettor with a pre-wetted 200 µL tip, add 50 µL of organoid/Matrigel® suspension to each of 4 wells of the warm 24-well plate such that it forms a dome in the middle of the well. Dispense to the first stop of the pipettor to avoid introducing bubbles.
- Place the lid on the culture plate. Incubate at 37°C and 5% CO<sub>2</sub> for 10 minutes to set the Matrigel® domes.
- Without disturbing the domes, add 750 µL of complete IntestiCult™ Organoid Growth Medium against the side of each well containing a dome. Do not pipette directly onto the domes.
- Add sterile PBS to any unused wells.
- Place the lid on the culture plate and incubate at 37°C and 5% CO<sub>2</sub>.
- Perform a full-medium change 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.

NOTE: For best results, passage organoids 2 times before cryopreservation or downstream experiments. Organoid growth will be slow at first, with spheroids forming within 1 - 2 days and budding after 5 - 7 days. Organoids should be ready for passaging after 5 - 7 days. After 1 - 2 passages, typical organoid growth characteristics should be restored. For passaging and cryopreservation protocols, refer to the Technical Bulletin: Intestinal Epithelial Organoid Culture with IntestiCult™ Organoid Growth Medium (Mouse; Document #28223), available at [www.stemcell.com](http://www.stemcell.com), or contact us to request a copy.



This product was developed under a license to intellectual property owned by Hubrecht Organoid Technology (HUB). This product is sold for research use only. Purchase of this product does not include the right to use it for drug screening aiming for commercial gain, equipment validation, biobanking, or for other commercial purposes. Purchasers wishing to use the product for purposes other than basic research use should contact HUB at [www.huborganoids.nl](http://www.huborganoids.nl) to obtain a further license. Purchasers may apply for a License from HUB, which will not be unreasonably withheld by HUB.

PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED. FOR ADDITIONAL INFORMATION ON QUALITY AT STEMCELL, REFER TO [WWW.STEMCELL.COM/COMPLIANCE](http://WWW.STEMCELL.COM/COMPLIANCE).

Copyright © 2025 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, and IntestiCult are trademarks of STEMCELL Technologies Canada Inc. Corning, Costar, and Matrigel are registered trademarks of Corning Incorporated. CryoStor is a registered trademark of BioLife Solutions. All other trademarks are the property of their respective holders. While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.