Mouse Intestinal Organoids

Cryopreserved mouse intestinal epithelial organoids for establishment of organoid cultures

Catalog #70931 200 Organoids

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

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) and cryopreserved in CryoStor® CS10.

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.

Properties

Storage: Store at -135°C or colder.

Shelf Life: Stable for 6 months from date of manufacture (MFG) on label.

Contains:
- Frozen mouse intestinal organoid segments
- CryoStor® CS10 (Catalog #07930)

Materials Required But Not Included

PRODUCT NAME	CATALOG #
IntestiCult™ Organoid Growth Medium (Mouse)	06005
DMEM/F-12 with 15 mM HEPES	36254
Bovine serum albumin (BSA)	---
Corning® Matrigel® Matrix, Growth Factor Reduced (GFR), Phenol Red-Free	Corning 356231
Costar® 24 Well Clear TC-Treated Multiple Well Plates	Corning 3526

Directions for Use

The following instructions are for preparing one cryovial of organoids for 4 wells of a 24-well plate.

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.

1. Thaw 120 µL of Matrigel® on ice.
2. Prepare complete IntestiCult™ Organoid Growth Medium (refer to the Product Information Sheet for IntestiCult™ [Document #28206]). 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 (15 - 25°C) for the duration of the protocol. Store the remaining DMEM + BSA at 2 - 8°C for up to 1 month.

5. 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.

6. 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.

7. Wipe the outside of the cryovial with 70% ethanol or isopropanol before opening.
8. Using a 1000 µL pipette, 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.
9. 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.
10. 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.
11. Using a 200 µL pipette, add 100 µL of complete IntestiCult™ Organoid Growth Medium to the tube. Using the same pipette tip, mix by pipetting up and down until organoids are resuspended, approximately 5 - 10 times.
12. Using a 200 µL pipette, 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.
13. Using a pipette 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 pipette to avoid introducing bubbles.
14. Incubate at 37°C and 5% CO_2 for 10 minutes to set the Matrigel® domes.
15. Add 750 µL of complete IntestiCult™ Organoid Growth Medium to each well containing a Matrigel® dome by pipetting the medium gently down the wall of the well. Do not pipette directly onto the domed cultures.
16. Add sterile PBS to any unused wells.
17. Place the lid on the culture plate and incubate at 37°C and 5% CO_2.
18. 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: 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 on our website at www.stemcell.com or contact us to request a copy.

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