User Guide:

Thawing and Plating Cryopreserved Intestine Epithelial Cells

Product Information

Intestine Epithelial Cells (IECs), cryopreserved at P1 and verified by representative markers such as ZO-1, form a critical, single-layered barrier lining the gut, directly interfacing with the luminal environment. This rapidly renewing epithelium, sustained by intestinal stem cells, differentiates into multiple specific cell types. These include nutrient-absorbing enterocytes, mucin-secreting goblet cells, antimicrobial Paneth cells, and hormone-releasing enteroendocrine cells. Together, they perform essential functions in digestion, immune surveillance, and microbial defense. Due to these central roles, IECs are fundamental models for studying drug absorption, inflammatory bowel disease, infection, and for developing advanced intestine organoid systems.

Cat. No.	Product Description	Size (Cells)
SD900-05	SD Rat Intestine Epithelial Cells	0.5 million
SD900-10	SD Rat Intestine Epithelial Cells	1 million
CD900-05	CD-1 Mouse Intestine Epithelial Cells	0.5 million
CD900-10	CD-1 Mouse Intestine Epithelial Cells	1 million
BA900-05	BALB/c Mouse Intestine Epithelial Cells	0.5 million
BA900-10	BALB/c Mouse Intestine Epithelial Cells	1 million
BD900-05	Beagle Dog Intestine Epithelial Cells	0.5 million
BD900-10	Beagle Dog Intestine Epithelial Cells	1 million
CY900-05	Cynomolgus Monkey Intestine Epithelial Cells	0.5 million
CY900-10	Cynomolgus Monkey Intestine Epithelial Cells	1 million
BM900-05	Bama Minipig Intestine Epithelial Cells	0.5 million
BM900-10	Bama Minipig Intestine Epithelial Cells	1 million

Storage & Shelf Life

Stable for 5 years at ≤ –150°C.

Thawing Protocol

1. The complete Intestine Epithelial Cell Medium should be pre-warmed to 37°C before use. For detailed instructions, please refer to ***Intestine Epithelial Cell Medium Kit Datasheet***.
   Plate/Dish/Flask Coating: Prior to thawing Intestine Epithelial Cells, it is recommended to pre-treat the surface of the cell culture plate/dish/flask with Collagen Type I from rat tail to enhance cell attachment.
2. Transfer 15 mL of pre-warmed complete Intestine Epithelial Cell Medium to a sterile 50ml centrifuge tube.
3. Take cryovial out of the liquid nitrogen (transport on dry ice or in liquid nitrogen).
4. Thaw cells for approx. 2 minutes at 37°C in the water bath. A portion of small ice crystals can be retained in the cryovial to prevent excessive incubation.
5. Shake gently during thawing. When the cells detach from the vial wall, transfer the content of vial into the complete Intestine Epithelial Cell Medium.
6. Add 1ml of complete Intestine Epithelial Cell Medium to the vial to wash any remaining cells from the vial (s).
7. Spin down at 300 x g for 5 minutes at room temperature to pellet the Intestine Epithelial Cells.
8. Carefully remove the supernatant without disturbing the pellet. Resuspend pellet in 1 mL of complete Intestine Epithelial Cell Medium.

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User Guide:

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9. Determine the total cell count and the number of viable cells using the Trypan Blue exclusion method.
10. Dilute the cells to the desired number of viable cells/mL (The appropriate cell density is assay dependent- recommended 4-6x10^4 cells/cm^2) with complete Intestine Epithelial Cell Medium.
11. Add an appropriate volume of diluted cells to collagen-coated cell culture plates as follows:

    6-Well plate: 2 mL/well (requires a total volume of 12 mL per 6-Well plate)

    12-Well plate: 1 mL/well (requires a total volume of 12 mL per 12-Well plate)

    24-Well plate: 0.5 mL/well (requires a total volume of 12 mL per 24-Well plate)

    48-Well plate: 0.2 mL/well (requires a total volume of 10 mL per 48-Well plate)

    96-Well plate: 0.1 mL/well (requires a total volume of 10 mL per 96-Well plate)
12. Gently shake the plates in a back-and-forth and side-to-side manner to evenly distribute the cells. Avoid any circular movement, as this will cause the cells to unevenly pool in the center of the plates.
13. Carefully place the plates into a 37°C, 5% CO_2, saturating humidity incubator to allow the cells to attach.
14. After 24h of cell adhesion, replace the pre-warmed complete Intestine Epithelial Cell Medium and proceed with the experiment. If necessary, replace the complete medium every 2-3 days.

Subculturing Protocol

1. Cells are ready for subculturing when they reach approximately 90% confluence.
2. Pre-warm 1× DPBS, 0.25% Trypsin and complete Intestine Epithelial Cell Medium to 37°C prior to use with the cells.
3. Aspirate and discard the culture media without disturbing the monolayer. Add pre-warmed sterile 1× DPBS (e.g., 3-5 mL for 6cm dish), gently swirl the plate/dish/flask to wash the cells, then aspirate and discard the DPBS.
4. Add pre-warmed 0.25% Trypsin (e.g., 1 mL for a 60mm dish), ensure it covers the surface evenly before transferring the plate/dish/flask to a 37°C, CO_2 incubator for 1-2 minutes.
5. Monitor the cells under a microscope. Digestion is complete when the cells detachy from the plate/dish/flask, round up and detach easily upon gentle agitation of the plate/dish/flask.
6. When the majority of cells are detached, quickly add pre-warmed complete Intestine Epithelial Cell Medium(e.g., 3 mL for 60mm dish) to terminate digestion. Gently pipette the solution to dislodge any remaining cells.
7. Transfer the dissociated cell suspensions to a sterile centrifuge tube, add 1× DPBS (e.g., 3 mL for a 60mm dish) to rinse the plate/dish/flask and collect any remaining cells.
8. Centrifuge the cells at 300 x g for 5 minutes at room temperature. After centrifugation, carefully aspirate and discard the supernatant.
9. Resuspend the cell pellet in 1mL pre-warmed complete Intestine Epithelial Cell Medium.
10. Count the cells and seed collagen-coated new plate/dish/flask at a density of 4-6x 10^4 cells/cm^2.
11. Place freshly seeded plate/dish/flask in a 37°C, 5% CO_2 incubator.
12. Allow the cells to adhere completely. Thereafter, replace the complete Intestine Epithelial Cell Medium every 2-3 days.

Cell Freezing Protocol

1. Cell Processing：Dissociate cells to be cryopreserved into suspension with pre-warmed 0.25% Trypsin. Rinse cells once with pre-warmed sterile 1× DPBS to remove the residual medium.

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2. Cell Counting: Cell counts are necessary in order to calculate the volume of Kryogene® Cell Freezing Media-Serum Free (AR0018-100) added for known cell numbers. Count the total cell number prior to cryopreservation to calculate the required volume of Kryogene® Cell Freezing Media-Serum Free.
3. Centrifuge cells to obtain a compact cell pellet.
4. Remove Supernatant: Remove the supernatant (typically residual buffer) as much as possible to minimize the dilution of Kryogene® Cell Freezing Media-Serum Free.
5. Add cold (2~8℃) Kryogene® Cell Freezing Media-Serum Free to resuspend the cells. Recommended Cell Density: 5~10×10^5 cells/mL.
   - Please mix the cell freezing media well prior to use.
   - Add Kryogene® Cell Freezing Media-Serum Free dropwise to minimize rapid change between intracellular and extracellular osmotic pressure, which may cause cell damage..
   - Pre-cool Kryogene® Cell Freezing Media-Serum Free at 2~8℃ prior to use.
6. Aliquot the cell suspension into cryotubes.
7. Pre-freeze Incubation: Incubate cell suspension at 2~8℃ for approximately 10 minutes prior to freezing.
8. Controlled Freezing: Freeze samples at -80℃ (±10℃).
   - Use a controlled rate freezer (-1℃/min) or similar protocol for most mammalian cell systems.
   - The freezing device or isopropanol container should be pre-cooled to 2~8℃ prior to use.
   - The recommended freezing duration at -80℃ using isopropanol containers is approximately 4 hours, with a maximum limit of overnight.
   - The controlled rate freezer should be validated to ensure optimal cryopreservation performance.
9. Cell Storage: Transfer cryovials to a liquid nitrogen environment for long-term storage.

Related Products

Cat. No.	Product Description	Size	Store at
IEpiC01-500	Intestine Epithelial Cell Medium Kit (IEpiCM)	500 mL/Kit	
IEpiC01-500b	Intestine Epithelial Cell Basal Medium (IEpiC-b)	500 mL	4℃
IEpiC01-GS-5	Intestine Epithelial Cell Growth Supplement (IEpiCGs)	for 500 mL	-20℃
FBS-50	Fetal Bovine Serum (FBS)	50 mL	-20℃
PS-5	Penicillin-Streptomycin (P/S)	5 mL	-20℃
Col-Coated-6w	Collagen I Coated 6-well Plate	1 Plate	4℃
Col-Coated-12w	Collagen I Coated 12-well Plate	1 Plate	4℃
Col-Coated-48w	Collagen I Coated 48-well Plate	1 Plate	4℃
Col-Coated-96w	Collagen I Coated 96-well Plate	1 Plate	4℃
Col-Coated-384w	Collagen I Coated 384-well Plate	1 Plate	4℃
AR0018-100	Kryogene® Cell Freezing Media-Serum Free	100 mL	4℃

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