Cat. # Y50035

For Research Use

TaKaRa

Cellartis® Intestinal Epithelial Cells (from ChiPSC18) Kit

Product Manual

v201908

Table of Contents

I. Description

Cellartis Intestinal Epithelial Cells (from ChiPSC18) are small intestinal epithelial cells derived from human iPS cells. These cells have intestinal epithelial cell-like characteristics, including the ability to form tight junctions and the expression of villin and the intestinal epithelial transcription factor CDX2. In addition, the cells have higher expression of the metabolic enzyme CYP3A4 and transporter PEPT1 compared to the human colon cancer Caco-2 cell line, allowing for better predictive results when evaluating metabolic absorption and drug interactions.

The Cellartis Intestinal Epithelial Cells (from ChiPSC18) Kit consists of a frozen vial of Cellartis Intestinal Epithelial Cells (from ChiPSC18)* and the Cellartis IEC Maturation Kit, which contains basal medium and supplements that are necessary for the cells' maturation. Following thawing, Cellartis Intestinal Epithelial Cells (from ChiPSC18) can be used for various assays after a 5-day pre-cultivation using the Cellartis IEC Maturation Kit.

* The Cellartis Human iPS Cell Line ChiPSC18 (Cat. # Y00305), cultured under feeder-free conditions using the Cellartis DEF-CS™ 500 Culture System (Cat. # Y30010), is used in the manufacturing of this product.

Other characteristics:

Tight junction formation in a short period of time

As this kit can induce the formation of tight junctions between cells within 5 days of cultivation after cell thawing, it is possible to efficiently assay for tight junctions and reduce experimental time as compared to Caco-2 cells, which normally require a 20-day cultivation period.

Stable quality

As these cells are produced from human iPS cells with unlimited proliferative capability using a highly reproducible differentiation induction method, they have an identical genetic background and low variance between lots as compared to primary human intestinal epithelial cells.

This product was jointly developed when Takara Bio received a technology transfer for a differentiation induction method for small intestinal epithelial cells derived from human iPS cells. This method was studied and developed by Dr. Hiroyuki Mizuguchi (Professor of Graduate School of Pharmaceutical Sciences, Osaka University, Project Reader of the National Institutes of Biomedical Innovation, Health and Nutrition) and Dr. Kazuo Takayama (Assistant Professor, Osaka University).

II. Components

Cellartis Intestinal Epithelial Cells (from ChiPSC18)	Frozen cells (1 vial; > 4.8 x 10^6 cells)	
Cellartis IEC Maturation Kit		
Cellartis IEC Maturation Basal Medium	1 bottle	100 ml
Cellartis IEC Supplement A	1 vial	100 µl
Cellartis IEC Supplement B	1 vial	100 µl
Cellartis IEC Supplement C	1 vial	50 µl
Cellartis IEC Supplement D	1 vial	40 µl

Materials required but not provided

- Tissue culture plates
- Cell culture inserts and companion plates (if doing air-liquid interface culture)
  Recommended: Falcon Cell Culture Inserts and Companion Plates (Corning), 6-well plate (Insert: Cat. # 353090, Plate: Cat. # 353502) or 12-well plate (Insert: Cat. # 353180, Plate: Cat. # 353503)
- PBS
- Coating reagent
  Matrigel Growth Factor Reduced (GFR) Basement Membrane Matrix, LDEV-Free, 10 ml (Corning, Cat. # 354230)
- DMEM (to dilute coating reagent)
- Trypan Blue solution
- Hemocytometer
- Ethanol (for disinfection)
- 37℃, 5% CO_2 incubator
- Clean bench or safety cabinet
- Centrifuge
- Microscope
- Water bath
- Electric pipette controller and plastic pipettes
- Micropipettor and sterilized tips (with filters)
- Centrifuge tubes

III. Storage

- After receiving this product, immediately store Cellartis Intestinal Epithelial Cells (from ChiPSC18) in liquid nitrogen.

- Store Cellartis IEC Maturation Kit (Basal Medium, Supplements A-D) at -20℃ until just before use. After thawing, store at 4℃ and use within 2 weeks (do refreeze).

- We recommend thawing Cellartis IEC Maturation Basal Medium at 4℃ overnight. When thawing at room temperature or in water at ambient temperature, be careful not to leave it for a long time. After thawing, mix well prior to use.

- Since Cellartis IEC Supplement B and IEC Supplement D contain DMSO, they may solidify at 4℃. They can be melted at room temperature in 5 - 10 minutes. Do not heat to melt them, and be careful not to leave them at room temperature for an extended period of time.

IV. Precautions for Cellartis IEC Maturation Kit

1. Avoid high temperatures, high humidity, ultraviolet light, and direct sunlight.
2. Do not leave this kit at room temperature for an extended period of time, as this can cause decreased performance.
3. The required amount of medium must be prepared just prior to use. Do not prepare it all at once.
4. Use the medium after aliquoting the required amount and warming it to between room temperature and 37℃. Add Supplements after warming Basal Medium.
5. It is not recommended to cryopreserve the prepared induction medium nor to refreeze (freeze and thaw) the Supplements.
6. The medium contains antibiotics, so it is not necessary to add additional antibiotics.
7. This product requires a separate coating reagent for cell culture plates.
8. The plating efficiency of the cells is 20 - 30% when cultured according to Section V. This will result in the appearance of unattached, floating cells 1 day after thawing the cells in the cryovial. However, there is no problem if attached cells are crowded or confluent in Step V-4. Perform a medium change on Day 1, and then seed the cells into the culture vessels according to Table 2.

V. Protocol

Please perform medium preparation and cell culture using aseptic technique in a safety cabinet or clean bench.

V-1. Preparation of Coating Reagent

- As only a small amount of Matrigel coating reagent is required, we recommend aliquoting and freezing the reagent. Thaw Matrigel at 4℃ overnight, dispense aliquots of the required amount (100 to 200 μl or more), and refreeze it. Do not repeat freezing and thawing after this.

  [Note] Matrigel forms a gel when heated, so perform aliquoting steps on ice. Keep tips, etc. at 4℃ in a refrigerator.

- Calculate the amount of coating reagent required, and then thaw at 4℃ either overnight or 1 - 2 hours before use.
- One day before use, thaw IEC Maturation Basal Medium overnight at 4℃. Mix well after thawing.

V-2. Coating of Culture Vessels: Day 0

1. Thaw aliquot(s) of Matrigel at 4℃.

   [Note] Matrigel forms a gel when heated, so prepare it on ice. Keep tips, etc. at 4℃ in a refrigerator.

2. Prepare the required amount (≧ 3 ml when using 1 vial of Cellartis Intestinal Epithelial Cells) of coating solution (Table 1) by diluting Matrigel with cold DMEM at a 1 : 50 ratio (Matrigel:DMEM). Mix well, and then add the coating solution to culture vessels and spread it evenly over the bottom of the culture vessel.

   Example: In order to prepare 4 ml of coating solution, add 80 μl of Matrigel to 3.92 ml of cold DMEM.

   Table 1. Amount of coating solution added to different culture vessels.

   For Cell Culture Inserts and Companion Plates

	6-well plate	12-well plate
Surface area/well	4.2 cm^2	0.9 cm^2
Coating solution/well	1 ml	0.25 ml

   For tissue culture plates

	12-well plate	24-well plate	48-well plate	96-well plate
Surface area/well	3.8 cm^2	1.9 cm^2	1.0 cm^2	0.35 cm^2
Coating solution/well	1 ml	0.5 ml	0.25 ml	0.1 ml

3. Incubate at 37℃ for >60 minutes.

V-3. Thawing Cells: Day 0

- Plating efficiency of Cellartis Intestinal Epithelial Cells (from ChiPSC18) decreases if not promptly seeded after thawing. Start the protocol with enough preliminary preparation so that cells can be seeded within 30 - 40 minutes after thawing.
- Cellartis Intestinal Epithelial Cells (from ChiPSC18) should be handled gently at pipetting and tapping steps.

1. Set a water bath to 37℃.
2. Dispense 9 ml of IEC Maturation Basal Medium for thawing cells in a centrifuge tube and warm to room temperature or 37℃. It is not necessary to add Supplements to this medium. This is used when thawing cells.
3. Dispense 4 ml of IEC Maturation Basal Medium in another tube, then warm to room temperature or 37℃. This will be for the preparation of induction medium.

   [Note] Avoid heating for long periods of time, as this can cause medium deterioration.
4. Add 4 µl (1 : 1,000 volume) each of IEC Supplement A and IEC Supplement B, and 16 µl (1 : 250 volume) of IEC Supplement C into the medium warmed up in Step 3, and then mix well.

   [Note] Add supplements after the medium is warmed.
5. Thaw a frozen cryovial of Cellartis Intestinal Epithelial Cells in a 37℃ water bath until only a small chip of ice remains (so that it melts and disappears during Step 6).

   [Note] A standard incubation at 37℃ takes 90 to 120 seconds. Be careful not to excessively incubate the cells when they are suspended in a cryopreservation agent.
6. Remove moisture from the outside of the vial with Kimwipes, then wipe with ethanol to disinfect.
7. Open the lid of the vial and gently transfer the cells into 9 ml of medium from Step 2.
8. Collect remaining cells by rinsing the inside of the vial with 1 ml of medium in the tube.
9. Centrifuge at 200*g* for 5 minutes at room temperature.
10. Remove supernatant, leaving about 0.2 ml. Loosen the cell pellet by gently tapping.
11. Add 2.5 ml of the induction medium prepared in Step 4, and then gently pipette up and down 3 to 4 times to mix. Measure the volume of the cell suspension.
12. After resuspending the cells, promptly take 20 µl for cell counting on the hemocytometer. Perform a cell count at a 1 : 8 or 1 : 10 dilution ratio using Trypan Blue solution. Calculate the number of cells and viable cell ratio.
13. Adjust the cell density to 1.6×10^6 cells/ml by adding medium.
14. After the culture vessel has incubated in coating solution for >60 minutes, remove the coating solution. Gently resuspend the cell suspension prepared in Step 13 by pipetting 3 to 4 times, and then seed the cells into the culture vessels (refer to Table 2.)

    [Note] When you use Cell Culture Inserts and Companion Plates, set the insert on the groove of the plate so that the insert does not tilt.
15. Incubate in a 37℃, 5% CO_2 incubator overnight, then change the medium the next day.

Table 2. Cell density to seed in different culture vessels.

For Cell Culture Inserts and Companion Plates

	6-well plate	12-well plate
Surface area/well	4.2 cm^2	0.9 cm^2
Medium volume/well	1 ml	0.25 ml
Cell number/well	1.6 x 10^6	4 x 10^5
Per 1 vial	3 wells	12 wells

For tissue culture plates

	12-well plate	24-well plate	48-well plate	96-well plate
Surface area/well	3.8 cm^2	1.9 cm^2	1.0 cm^2	0.35 cm^2
Medium volume/well	1 ml	0.5 ml	0.25 ml	0.1 ml
Cell number/well	1.6 x 10^6	8 x 10^5	4 x 10^5	1.6 x 10^5
Per 1 vial	3 wells	6 wells	12 wells	30 wells

V-4. Medium Change: Day 1

- Change medium at Day 1 after seeding. For optimal results, change medium within 15 - 24 hours after seeding.
- While there will be unattached floating cells at Day 1 after thawing, there is no problem if attached cells are crowded or confluent.

1. Dispense 4 ml of IEC Maturation Basal Medium and warm it up to between room temperature and 37℃.
2. Prepare induction medium by adding 4 µl (1 : 1,000 volume) of IEC Supplement A and IEC Supplement B, and 2 µl (1 : 2,000 volume) of IEC Supplement D. Mix well.
3. There will be a large amount of unattached floating cells the day after thawing. Remove as many floating cells as possible when you change the medium. Gently shake the plate to diffuse the floating cells and then aspirate cell culture medium. Promptly add new induction medium (refer to Table 2).

   [Note] When you use Cell Culture Inserts and Companion Plates, be careful to not damage cells and membranes of Cell Culture Inserts with suction.

4. Continue cultivating in a 37℃, 5% CO_2 incubator.

V-5. Medium Changes: After Day 3

Perform a medium change every other day (refer to Table 3).

1. Dispense 4 ml of IEC Maturation Basal Medium and warm to between room temperature and 37℃.
2. Prepare the induction medium by adding 4 μl (1 : 1,000 volume) of IEC Supplement A and IEC Supplement B. Mix well.
3. Aspirate culture medium from the vessels and promptly add new induction medium (refer to Table 2).

   [Note] When you use Cell Culture Inserts and Companion Plates, be careful to not damage cells and membranes of Cell Culture Inserts with suction.

4. Continue to culture in a 37℃, 5% CO_2 incubator.

   [Note] Assays can be run between the 5th and 8th day of culture (on Days 5, 6, 7, and 8). Although cell culture can be performed after the 8th day, it is not recommended to run assays using the cells after Day 8.

Table 3. Maturation schedule

Day 0	Thawing and seeding, IEC Supplements A + B + C	
Day 1	Medium change, IEC Supplements A + B + D	
Day 2		
Day 3	Medium change, IEC Supplements A + B	
Day 4		
Day 5	Medium change, IEC Supplements A + B	Available for assay
Day 6		
Day 7	Medium change, IEC Supplements A + B	
Day 8		

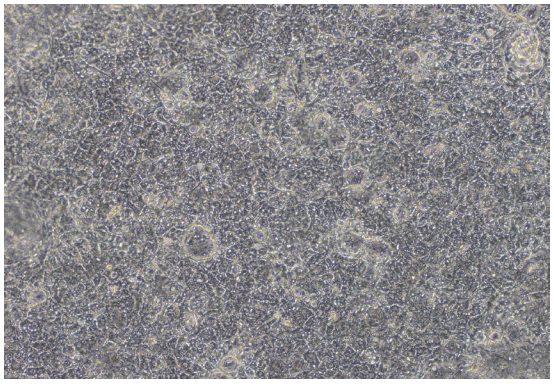

Figure 1. Cells on the 8th day of culture.

VI. Related Products

Cellartis® Human iPS Cell Line 18 (ChiPSC18) Kit (Cat. # Y00305)
Cellartis® Definitive Endoderm Cells (from ChiPSC18) (Cat. # Y10040)
Cellartis® Enhanced hiPS-HEP v2 (from ChiPSC12) Kit (Cat. # Y10133)
Cellartis® Enhanced hiPS-HEP v2 (from ChiPSC18) Kit (Cat. # Y10134)
Cellartis® Enhanced hiPS-HEP v2 (from ChiPSC22) Kit (Cat. # Y10135)
Cellartis® hiPS Beta Cells (from ChiPSC12) Kit (Cat. # Y10100)
Cellartis® hiPS Beta Cells (from ChiPSC22) Kit (Cat. # Y10106)
MiraCell® Cardiomyocytes v2 (from ChiPSC12) Kit (Cat. # Y50025)*
MiraCell® Endothelial Cells (from ChiPSC12) Kit (Cat. # Y50055)*

* Not available in all geographic locations. Check for availability in your area.

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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