Cat. # Y50045

For Research Use

TaKaRa

Cellartis® Microglia (from ChiPSC12) Kit

Product Manual

v202010

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I. Description

Recent studies have reported that microglia, a type of glial cell, play multiple roles in the central nervous system's immune response and homeostasis. These functions include the protection, new formation, and lesion restoration of nerve tissue, etc. On the other hand, when there is continuous excessive activation, it is also involved in neurodegenerative diseases, such as Alzheimer's and Parkinson's.

Cellartis Microglia (from ChiPSC12) are microglia-like cells prepared from the human iPS cell line (ChiPSC12) by a differentiation induction method wherein the development of yolk-sac macrophages and microglia is recreated *in vitro*. The cells show characteristics similar to microglia derived from a living human body, such as a high expression of microglia marker (Iba1) and cell morphology with projection structures. In addition, the cells also have functions that primary microglia possess, such as phagocytosis of amyloid β protein, migration in response to ATP, and cytokine release in response to lipopolysaccharides.

This product includes a frozen vial of Cellartis Microglia (from ChiPSC12) and Cellartis Microglia Culture Medium, with which highly functional microglia-like cells can be prepared.

Cellartis human iPS cell line 12 (ChiPSC12) (sold as a part of Cat. #Y00285),* cultured under feeder-free conditions with the Cellartis DEF-CS™ 500 Culture System (Cat. #Y30010), is used in the manufacturing of Cellartis Microglia (from ChiPSC12).

* Please refer to the following website regarding the details of ChiPSC12.
https://www.takarabio.com/products/stem-cell-research/stem-cells-and-stem-cell-derived-cells/human-induced-pluripotent-stem-cells

Takara Bio Inc. makes and sells this product using a manufacturing method for deriving microglia from human iPS cells that is based on the acquisition of an exclusive worldwide license from Shionogi & Co., Ltd.

II. Components

Cellartis Microglia (from ChiPSC12)	Frozen cells (1 vial; >1 x 10^6 cells) (≥ 8 x 10^5 viable cells)	
Cellartis Microglia Culture Medium		
Cellartis Microglia Culture Basal Medium	1 bottle	50 ml
Cellartis Microglia Culture Supplement	1 vial	5 ml

Materials Required but not Provided

- 37℃, 5% CO_2 incubator
- Clean bench or safety cabinet
- Centrifuge
- Microscope
- Water bath
- Pipette controller and plastic pipettes
- Micropipettor and sterilized tips (with filters)
- Centrifuge tubes
- Cell culture vessels
  * The following are culture vessels which have been used successfully at our company.
    - Corning BioCoat Poly-D-Lysine/Laminin 96-well Clear Flat Bottom TC-treated Microplate (Corning: Code. 354596), recommended
    - Corning BioCoat Poly-D-Lysine 96-well Clear Flat Bottom TC-treated Microplate (Corning: Code. 354461)
- DNase I, Bovine Pancreas（MERCK: Code. 260913）
- DPBS（Dulbecco's PBS)
- Sterilization filter（Millex-GV Syringe Filter Unit, 0.22 μm, PVDF, 33 mm, gamma sterilized [MERCK: Code. SLGVR33RS, etc.])
- Trypan blue solution
- Hemocytometer
- Ethanol (for disinfection)

III. Storage

- After receiving this product, immediately store the vial containing Cellartis Microglia (from ChiPSC12) in liquid nitrogen.
- Store Cellartis Microglia Culture Basal Medium at 4℃. Do not freeze.
- Store Cellartis Microglia Culture Supplement at -20℃.

IV. Precautions Before Use

- Avoid high temperature, high humidity, ultraviolet light, and direct sunlight.
- Store Cellartis Microglia Culture Medium at 4℃ after preparation. Do not leave it at room temperature for an extended period of time, as this can cause decreased performance.
- Prepare Cellartis Microglia Culture Medium on the day Cellartis Microglia (from ChiPSC12) is defrosted and use it within one week after preparation.
- Do not refreeze Cellartis Microglia Culture Supplement after thawing.
- Dispense only the required volume of Cellartis Microglia Culture Medium. Warm the dispenced medium between room temperature and 37℃. Avoid warming up the entire volume. Also, do not freeze it for preservation.
- Cellartis Microglia Culture Basal Medium and Cellartis Microglia Culture Supplement do not contain antibiotics. Penicillin (100 units/ml) and streptomycin (100 μg/ml) can be added for culture, though effect of antibiotics on various assays has not been confirmed. Plates coated with poly-D-lysine/laminin or poly-D-lysine are recommended for culturing Cellartis Microglia (from ChiPSC12) after cell thawing. In addition, depending on the purpose of use, other examinations may be required to determine an optimal coating reagent.
- The product contains animal-derived components. When you dispose of it, perform an inactivation treatment such as autoclaving, following the guidelines provided by your institution.

V. Protocol

Perform medium preparation and cell culture using aseptic techniques in a safety cabinet or on a clean bench.

V-1. Preparation of Cellartis Microglia Culture Medium

1. Thaw Cellartis Microglia Culture Supplement at room temperature or 4℃ (overnight is acceptable).

   [Note] Use promptly after thawing. Do not leave Cellartis Microglia Culture Supplement at room temperature for a long time.

2. Remove 5 ml of medium from the bottle of Cellartis Microglia Culture Basal Medium.

   [Note] The removed medium, which does not contain animal derived material, may be used for various cell assays.

3. Add the entire amount of thawed Cellartis Microglia Culture Supplement into the Basal Medium bottle in Step 2 and mix thoroughly. You will have 50 ml of prepared Cellartis Microglia Culture Medium.

   [Note] Use the prepared Cellartis Microglia Culture Medium within one week.

V-2. Preparation of DNase I Stock Solution

1. Make the stock solution by dissolving DNase I in water.

   [Example] Dissolve DNase I, Bovine Pancreas（MERCK: Code. 260913-10MUCN) in 167 ml of water to prepare 60,000 Dornase units/ml of the stock solution.

2. Sterilize the DNase I solution with 0.22 μm syringe filter.

   [Note] Sterilized DNase I solution can be stored at -20℃ up to 3 months.

V-3. Thawing Cells

- A culture method using a 96-well plate is shown here as a reference.
- Plating efficiency of the Cellartis microglia decreases if they are not promptly seeded after thawing. Start the protocol with enough preliminary preparation so that cells can be seeded within 60 minutes after thawing.
- Do not use the outer wells of the 96-well plate for cell culture. Add sterile distilled water or DPBS in the outer wells to prevent evaporation.

1. Set water bath to 37℃.
2. Dispense 3 ml of Cellartis Microglia Culture Medium for cell culture in a sterilized tube, and warm it up to 37℃. This medium will be used for the dilution and initial seeding of the cells.

   [Note] Avoid heating for long periods of time, as it can cause the medium to deteriorate.
3. Dispense 9 ml of Cellartis Microglia Culture Medium in a 15-ml tube for thawing the cells, and warm it up to 37℃.
4. Just before thawing the cells, add 10 µl of DNase I stock solution into the warmed 9 ml of medium in the 15-ml tube and mix it.
5. Thaw the frozen vial of cells in a 37 ℃ water bath until only a small chip of ice remains.

   [Note] A standard incubation takes 90 to 120 seconds. Be careful not to incubate the cells at 37 ℃ longer than 120 seconds.
6. Remove moisture from the outside of the vial with Kimwipes, and wipe with ethanol to disinfect.
7. Take 1 ml of Cellartis Microglia Culture Medium from the 15-ml tube prepared in Step 4, add it to the vial containing the cells, and very gently resuspend. This prevents the separation of cryopreservation agent and the medium at a later centrifugation. Then, transfer the cell suspension into the 15-ml tube prepared in Step 4, and let it sit at 37℃ for 10 minutes.

   [Note] DNase I degrades the DNA extruded from the dead cells, preventing them from clumping and pelleting during the centrifugation in the next step.
8. Centrifuge the 15-ml tube containing the cells at 200*g* for 5 minutes at room temperature.
9. Remove the supernatant, leaving about 50 µl. Loosen the pellet by gently tapping.
10. Add 0.7 ml of Cellartis Microglia Culture Medium (from Step 2) and gently resuspend the cell pellet.
11. Promptly remove about 20 µl of the cell suspension and count the cells using a hemocytometer (or auto cell counter, etc.) after diluting at a 1:4 ratio using trypan blue solution.
12. Adjust the cell density to 1 x 10^6 cells/ml by adding Cellartis Microglia Culture Medium prepared in Step 2.
13. Prior to seeding, add 40 µl of Cellartis Microglia Culture Medium to each well of a poly-D-lysine/laminin coated 96-well microplate. Add 60 µl of cell suspension (1 x 10^6 cells/ml) into each well and gently mix it twice with the medium in the well. Seeding density is 1.9 x 10^5 cells/cm^2 (surface area 0.32 cm^2).
14. Incubate in a 37℃, 5% CO_2 incubator.

V-4. Medium Change

- Perform the medium change gently using a pipette, as shown in Figure 1.
- Do not use an aspirator to remove the medium.
- Add the medium gently along the wall of the well.
- Perform the medium change following the culture schedule in Table 1.
- After thawing the cells, perform a medium change within 3 to 18 hours after starting culture in a CO_2 incubator.

[Medium change after thawing]

1. Dispense the required amount of Cellartis Microglia Culture Medium and warm it up to 37℃.
2. There will be a large amount of unattached, floating cells the day after thawing. Remove as many non-attached and dead cells as possible when you change the medium. Gently resuspend the culture supernatant twice and then remove the supernatant using a micropipette.
   [Note] Tilt the pipette tip so that the liquid flows toward the side of the well, not toward the bottom (Figure 1, left, is correct).

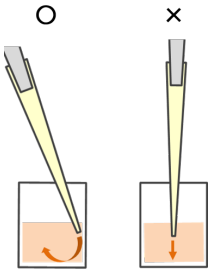

Figure 1. Recommended method to resuspend culture supernatant using a micropipette. Do not pipette directly downward.

3. Promptly and gently add new Cellartis Microglia Culture Medium along the wall of the well.

[Medium change on and after Day 3]

1. Dispense the required amount of Cellartis Microglia Culture Medium and warm it up to 37℃.
2. Remove culture supernatant using a micropipette, and promptly and gently add new Cellartis Microglia Culture Medium along the wall of the well (refer to Table 1 for volumes). Do not resuspend the cells while adding new medium.
3. Place the plate in a 5% CO_2 incubator at 37℃.

Table 1. Culture schedule and medium change method

	Culture step	Medium removed	Fresh medium	Total medium
Day 0	Thawing and seeding	-	100 µl	100 µl
3 to 18 hours after seeding	Whole medium change	100 µl	100 µl	100 µl
Day 2	-	-	-	-
Day 3	Partial medium change	50 µl	100 µl	150 µl
Day 4	-	-	-	-
Day 5	Partial medium change	100 µl	100 µl	150 µl
Day 6	-	-	-	-
Day 7	Partial medium change	100 µl	100 µl	150 µl

V-5. Experimental Example

[Cell morphology of Cellartis Microglia (from ChiPSC12) (Days 1, 2, 4, and 8)]

Cellartis Microglia (from ChiPSC12) were cultured using the protocol provided in this user manual for 8 days post-thawing.

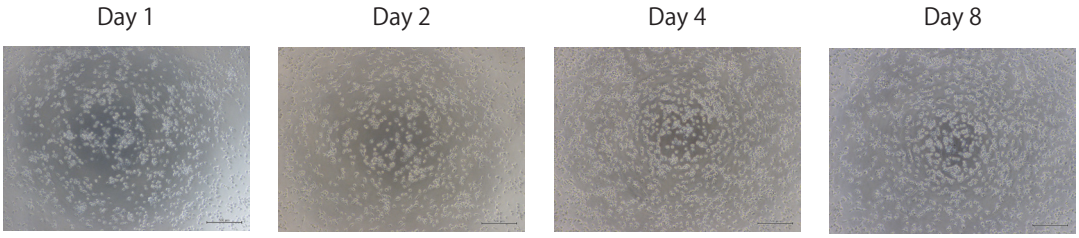

Figure 2. Cell morphology of Cellartis Microglia (from ChiPSC12).

VI. Related Products

Cellartis® human iPS cell line 12 (ChiPSC12) Kit (Cat. #Y00285)
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)*
Cellartis® Intestinal Epithelial Cells (from ChiPSC18) Kit (Cat. #Y50035)
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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