Cat. # Y50055

For Research Use

TaKaRa

MiraCell®
Endothelial Cells
(from ChiPSC12) Kit

Product Manual

v201801

Table of Contents

I. Description

Primary human vascular endothelial cells are widely used in research fields such as angiogenesis and drug uptake. The properties of commercially available human endothelial cells vary between different lots due to limitations on obtaining cells from a single donor. Vascular endothelial cells differentiated from human iPS cells have attracted attention as a substitute for primary human vascular endothelial cells because they are homogeneous cell populations derived from the same donor, due to the ability of iPS cells to proliferate indefinitely.

MiraCell Endothelial Cells (from ChiPSC12) are high-purity vascular endothelial cells differentiated from human iPS cells, which are available for analyzing the characteristics and function of endothelial cells, and for performing toxicity tests.

This product was jointly developed by Takara Bio Inc. and iHeart Japan Corporation, after Takara Bio Inc. introduced a production technique for human endothelial cells developed by Professor Jun K. Yamashita of the Center for iPS Cell Research and Application of Kyoto University, from iHeart Japan Corporation, in June 2014. This method makes it possible to prepare high-purity endothelial cells without using antibiotic or metabolic selection. In addition, this technique prevents the reduction in cell population purity that typically results from long-term cell culture.

The Cellartis® human iPS cell line 12 (ChiPSC12) Kit (Cat. #Y00285)*, cultured using the Cellartis DEF-CS™ 500 Culture System (Cat. #Y30010), is used in the manufacturing process for this product.

* http://catalog.takara-bio.co.jp/product/basic_info.php?unitid=U100009146

Product features

- More than 95% purity (CD31-positive)
- Purified without antibiotic and metabolic selection
- Endothelial tube formation ability
- Dil-Ac-LDL uptake activity
- Expresses a wide variety of endothelial cell genes

II. Components

MiraCell Endothelial Cells (from ChiPSC12)	Frozen vial, 1 tube	>1.5 x 10^6 cells
MiraCell EC Culture Medium		500 ml
MiraCell EC Culture Supplement		9 ml x 2

Necessary reagents and equipment not supplied in this kit

- 37℃ water bath
- 37℃, 5% CO_2 incubator
- Clean bench or safety cabinet
- Refrigerated centrifuge
- Pipettor and plastic pipets
- Micropipettor and sterilized tips (with filter)
- 50 ml centrifuge tubes
- 15 ml centrifuge tubes
- Dulbecco's PBS with Ca^{++} & Mg^{++} (D-PBS (+/+)
- Dulbecco's PBS without Ca^{++} & Mg^{++} [D-PBS (-/-)]
- Accumax Cell Dissociation Solution (Innovative Cell Technologies, Inc., Cat. #AM105)
- Cell culture vessels (6-well tissue culture plates, etc.)
- 1 mg/ml fibronectin solution (from human plasma; Sigma-Aldrich, Cat. #F0895 or equivalent)
- Trypan blue solution (0.4% trypan blue solution, Thermo Fisher Scientific, Cat. #15250-061 or equivalent)
- Hemocytometer

III. Storage

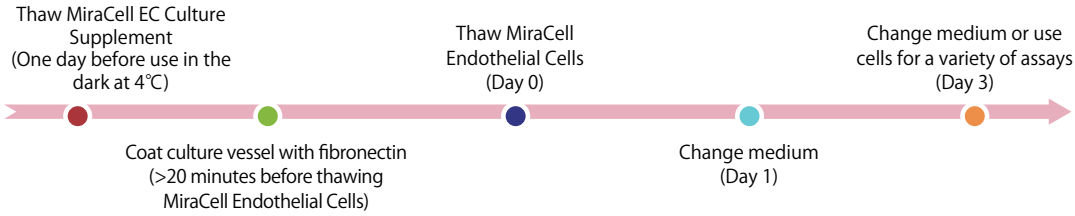
	Storage
MiraCell Endothelial Cells (from ChiPSC12)	Upon receipt, immediately store in liquid nitrogen.
MiraCell EC Culture Medium	Upon receipt, store at 4℃, protected from light. Do not freeze.
MiraCell EC Culture Supplement	Store frozen at -20℃ or less until one day before use. Do not refreeze after thawing.

IV. Preparation Before Use

1. To prevent culture medium inactivation, remove an aliquot of the amount needed and allow it to warm to room temperature.
2. Avoid warming up the culture medium for more than 1 hour.
3. Perform aseptic procedures on a clean bench or in a safety cabinet.
4. MiraCell EC Culture Medium does not contain antibiotics. When adding antibiotics, the recommended concentrations are 10 U/ml penicillin and 10 μg/ml streptomycin.

V. Protocol

V-1. Cell Culture Timeline



V-2. Preparation of MiraCell EC Culture Medium (+ Supplement)

[Prepare medium in advance before the cells are thawed]

1. Begin thawing MiraCell EC Culture Supplement in the dark at 4℃ the day before.
2. Prepare MiraCell EC Culture Medium (+ Supplement) by adding two tubes of thawed MiraCell EC Culture Supplement to 500 ml of MiraCell EC Culture Medium.
3. Use about 5 ml of MiraCell EC Culture Medium (+ Supplement) to rinse the MiraCell EC Culture Supplement tubes and add the rinsed medium to the bottle of MiraCell EC Culture Medium.

* Store the prepared medium in the dark at 4℃. Use MiraCell EC Culture Medium (+ Supplement) within 2 weeks after preparation. Do not freeze.

* A half volume of MiraCell EC Culture Medium (+ Supplement) can be prepared by transferring half of the MiraCell EC Culture Medium to another sterilized container, then adding only one tube of MiraCell EC Culture Supplement. Do not refreeze MiraCell EC Culture Medium (+ Supplement) after preparation.

V-3. Coating Culture Vessels with Fibronectin

[Note] Endothelial Cells can be spread onto about 15–18 wells of 6-well plates.

[Prepare 20 minutes before thawing endothelial cells]

1. Dilute 1 mg/ml fibronectin solution 20X with D-PBS (+/+) to a final concentration of 50 μg/ml.

D-PBS (+/+)	19 ml
1 mg/ml fibronectin solution	1 ml

2. Mix gently by pipetting.

   [Note] Avoid intense mixing such as vortexing etc., as it affects fibronectin activity.

3. Add 50 μg/ml fibronectin solution (0.1 ml/cm^2) to a culture vessel and spread the solution to cover the entire surface of the vessel.

Culture vessel	Diluted fibronectin
24-well plate	0.25 ml/well
12-well plate	0.5 ml/well
6-well plate	1.0 ml/well

4. Incubate the plate for >20 minutes in a 5% CO_2 incubator at 37℃.

   [Note] Remove the fibronectin solution just before adding the cell suspension.

V-4. Thawing and Culturing Endothelial Cells

[Day 0]

- Prepare a 37℃ water bath before thawing the cells.
- Transfer 8 ml of MiraCell EC Culture Medium (+ Supplement) to a 15-ml tube.
- Transfer about 35 ml of MiraCell EC Culture Medium (+ Supplement) required for cell culture to a 50-ml tube and warm it up to room temperature.
- Transfer a frozen vial of MiraCell Endothelial Cells (from ChiPSC12) from liquid nitrogen storage to a container with liquid nitrogen or dry ice.

1. Incubate the frozen vial for 2 minutes in a 37℃ water bath to thaw it.
   [Note] Do not shake the vial while it is thawing.
2. Sterilize the outside of the vial with 70% ethanol and remove any extra ethanol.
3. Using a 1 ml micropipettor, transfer the cell suspension from the vial to 8 ml of MiraCell EC Culture Medium (+ Supplement) in the 15-ml tube.
4. Rinse the vial with 1 ml of 35 ml of MiraCell EC Culture Medium (+ Supplement) that was set aside for cell culture, and then transfer the rinse to the 15-ml tube from Step 3.
5. Centrifuge at 200*g* at 20℃ for 5 minutes.
6. Remove the supernatant with an aspirator.
   [Note] Make sure to leave behind about 0.2 ml of supernatant, since removing all of the supernatant may result in loss of cells.
7. Loosen the cell pellet by gentle tapping.
8. Add 2 ml of MiraCell EC Culture Medium (+ Supplement) and pipette up and down 2 to 3 times to resuspend the pellet.
9. Take a 20 μl aliquot of the cell suspension, stain it with trypan blue solution, and count the number of cells using a hemocytometer (or automated cell counter).
10. Adjust the cell density to 0.5 x 10^5 cells/ml by adding an appropriate amount of MiraCell EC Culture Medium (+ Supplement).
11. Just after removing the fibronectin solution from the culture plate prepared in V-3. 4, add 2 ml of the cell suspension to one well of a 6-well plate. (For other culture vessels, see the table below.)

Culture vessel	Volume of cell suspension (0.5 x 10^5 cells/ml)	Cell number/well
24-well plate	0.5 ml	0.25 x 10^5
12-well plate	1 ml	0.5 x 10^5
6-well plate	2 ml	1.0 x 10^5

12. After evenly dispersing the cells by gently shaking the plate from right to left, incubate the cells in a 5% CO_2 incubator at 37℃.
    [Note] Avoid moving the culture vessel after putting it in the incubator, since doing so may result in uneven cell density and reduced plating efficiency.

[Day 1]

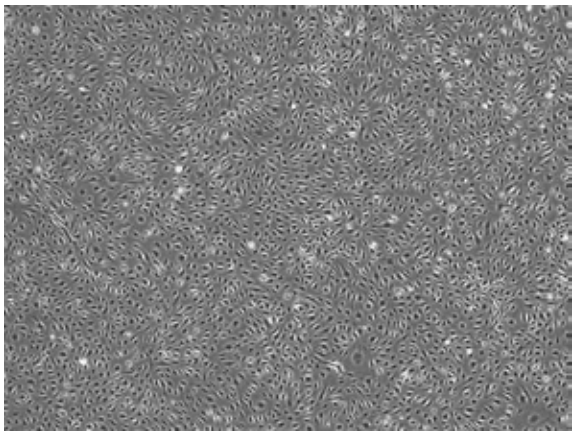
13. Aspirate off the culture medium from the culture vessel the next day (one day after the cells were inoculated).
14. Add MiraCell EC Culture Medium (+ Supplement) pre-warmed to room temperature beforehand.

Culture vessel	Volume of medium to be added
24-well plate	0.5 ml/well
12-well plate	1.0 ml/well
6-well plate	2.0 ml/well

15. Culture the cells for 2 more days in a 5% CO_2 incubator at 37℃. Then perform a medium change every other day or every 3 days, aspirating all the medium and adding the amount of pre-warmed MiraCell EC Culture Medium (+ Supplement) as indicated in the table above.

V-5. Subculturing Endothelial Cells

MiraCell Endothelial Cells (from ChiPSC12) grow confluent from 3 to 5 days after plating (refer to the lower figure), making it possible to subculture the cells into various types of culture vessels for assays. A method for subculturing cells into a 6-well plate is shown as an example.



1. Aspirate off the medium.
2. Add 2 ml of pre-warmed D-PBS (-/-) to each well of a 6-well plate.
3. After removing the D-PBS (-/-), add 2 ml of D-PBS (-/-) to each well again.
4. Remove the D-PBS (-/-) , then add 0.5 ml of Accumax Cell Dissociation Solution to each well, and incubate at 37℃ for 4 to 8 minutes.
5. Tap the plate sharply on the side to detach the endothelial cells as completely as possible.
6. Add 0.5 ml of pre-warmed MiraCell EC Culture Medium (+ Supplement) to each well and transfer the cell suspension in each well to a separate centrifuge tube using a 1 ml pipette.
7. Detach and recover the remaining cells using 1 ml of MiraCell EC Culture Medium (+ Supplement) by slowly pipetting 3 to 5 times at the upper and lower ends of each well.
8. Transfer the cell solution from each well to the corresponding centrifuge tube from Step 6.
9. After pipetting the cell suspensions once, take 20 μl aliquots of each cell suspension and count the number of cells.
10. Centrifuge the tubes at 200*g* at 20℃ for 5 minutes and remove the supernatants with an aspirator.
11. Resuspend the cells in each tube using MiraCell EC Culture Medium (+ Supplement) or an appropriate medium.
12. Plate the required number of cells in the culture vessel of your choice for assays. For extended cell cultures, using a plate coated with fibronectin is recommended.

VI. Experimental Example: Tube Formation

It is possible to perform a tube formation 3 days after thawing the cells.

1. Coat a plate using Matrigel Matrix (Corning, Cat. #354230) as follows:
   Thaw the Matrigel Matrix on ice, quickly add the amount indicated below to each well of a culture vessel, spread the solution to cover the entire surface of the vessel, and incubate it at 37℃ for >30 minutes.
   [Note] Use an undiluted solution of Matrigel Matrix. Avoid raising the temperature of Matrigel Matrix because it tends to solidify at higher temperatures.

Culture vessel	Amount of Matrigel
48-well plate	150 µl
24-well plate	300 µl

2. Collect the cells according to the procedure in Section V-5, and count the number of cells.
3. Centrifuge at 200*g* at 20℃ for 5 minutes and remove supernatant with an aspirator.
4. Resuspend the cells at a concentration of 3 x 10^5 cells/ml using the Endothelial Cell Growth Medium 2 Kit (PromoCell, Cat. #C-22111).
   [Note] The Endothelial Cell Growth Medium 2 Kit is recommended for use in endothelial cell tube formation in order to obtain more reproducible results. MiraCell EC Culture Medium can be also used for tube formation experiments by adjusting the cell density to 5 x 10^5 cells/ml.
5. Add the cell suspension slowly to the plate coated with Matrigel Matrix, along the wall of the well.
   [Note] Incubate the plate at 37℃ just before use. Do not remove Matrigel.

Culture vessel	Amount of cell suspension
48-well plate	100 µl
24-well plate	200 µl

6. Culture the cells overnight in a CO_2 incubator at 37℃ and observe them the next day.

VII. Related Products

Cellartis® human iPS cell line 12 (ChiPSC12) Kit (Cat. #Y00285)
MiraCell® EC Culture Medium (Cat. #Y50053)*
MiraCell® Cardiomyocytes (from ChiPSC12) Kit (Cat. #Y50015)*

* 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.

Takara products may not be resold or transferred, modified for resale or transfer, or used to manufacture commercial products without written approval from TAKARA BIO INC.

If you require licenses for other use, please contact us by phone at +81 77 565 6973 or from our website at www.takara-bio.com.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product web page. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.