Cat. # Y40031

For Research Use

TaKaRa

Cellartis® 2i mES/iPSC Culture Medium

Product Manual

v202002

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I. Description

Cellartis 2i mES/iPSC Culture Medium is a serum-free, defined cell culture medium for establishment of mouse ES and iPS cell lines containing two selective small molecule inhibitors, CHIR99021 and PD0325901 ('2i'), that act to eliminate differentiation-inducing signals from GSK3β and ERK/MEK, respectively, and promote cell proliferation.
Addition of Leukemia Inhibitory Factor (LIF) can also induce the efficient conversion of partial- or pre-iPS mouse cells into fully pluripotent iPS cells via Nanog[1, 2]. The culture medium can also be used for long-term maintenance of naïve, 'ground state' mouse pluripotent stem cells.
The 2i technology has also been used to establish ES cells from mouse strains for which it is difficult to produce germline-competent ES cells[3], and to induce iPS cells from human and livestock species[4, 5].

II. Components

Cellartis mES/iPSC Culture Basal Medium:	200 ml (Cat. #Y40005; not sold separately)
Cellartis 2i mES/iPSC Supplement:	200 μl (Cat. #Y40032; not sold separately)

III. Storage

Cellartis mES/iPSC Culture Basal Medium:	-20℃ (Do not refreeze after thawing.)
Cellartis 2i mES/iPSC Supplement:	-80℃ (Do not refreeze after thawing.)

IV. Precautions

1. Avoid exposure to high temperature, high humidity, ultraviolet light, and sunlight.
2. Use within 2 weeks after preparing Cellartis 2i mES/iPSC Culture Medium.
3. Store the prepared Cellartis 2i mES/iPSC Culture Medium at 4℃. Do not keep it at room temperature (RT) for a long time.
4. Before using the prepared Cellartis 2i mES/iPSC Culture Medium, dispense the amount, and then warm this aliquot to between room temperature (RT) and 37℃.

V. Materials Required but not Provided

- 37℃, 5% CO_2 incubator
- Clean bench or biosafety cabinet
- Centrifuge
- Microscope
- Water bath
- -80℃ deep freezer
- Liquid nitrogen storage tank or -150℃ deep freezer
- Freezing container (e.g., BICELL, Mr. Frosty)
- Electric pipet controller and plastic pipets
- Micropipette and sterilized tips (with filters)
- Centrifuge tubes
- Tissue culture coated cell culture vessels
- Cryovials
- D-PBS (-/-)
- Cell detachment reagent
  Accutase (e.g., Thermo Fisher Scientific, Cat. #A11105-01)
- Cryopreservation solution
- Trypan blue solution
- Hemocytometer
- 70% ethanol for disinfection
- Kimwipes
- Poly-L-ornithine hydrobromide
- Laminin
- Gelatin type A (optional)

VI. Protocol

This protocol is just an example. Please adjust it to the cell strains you use.
Use aseptic technique and a clean surface (such as a clean benchtop or biosafety cabinet) for all steps in this protocol.

VI-1. Preparation of Cellartis 2i mES/iPSC Culture Medium

1. Thaw Cellartis mES/iPSC Culture Basal Medium in a water bath (37℃) while protecting it from the light, and remove it from the water bath just before the medium has completely thawed (i.e., do not allow the media to warm up). Then, mix the medium gently and thaw completely.

   Alternatively, thaw the medium at 4℃ while protecting it from the light. If a precipitate appears in the medium, leave it at 4℃ overnight to completely dissolve the precipitate. Do not use media with visible precipitate; ensure it is dissolved before use.
2. Thaw Cellartis 2i mES/iPSC Supplement at room temperature (for no longer than 30 minutes) while protecting it from the light. Centrifuge briefly. Using sterile technique, add all of the supplement to thawed Cellartis mES/iPSC Culture Basal Medium and mix thoroughly. Do not filter sterilize.

VI-2. Coating of Cell Culture Vessels

1. Add 0.01% poly-L-ornithine hydrobromide in D-PBS (-/-) to the cell culture vessels (use 0.1 ml/cm^2 vessel area; see Table 1), making sure the entire surface is covered, and incubate at 37℃ ± 1℃ for 30 minutes or more.
2. Aspirate the solution from the cell culture vessels and wash twice with 2 volumes of D-PBS (-/-).
3. Add 10 μg/ml laminin in D-PBS (-/-) to the cell culture vessels (use 0.1 ml/cm^2 vessel area; see Table 1), making sure the entire surface is covered, and incubate at 37℃ ± 1℃ for 3 hours or more.
4. Aspirate the solution from the cell culture vessels and add D-PBS (-/-). Remove D-PBS (-/-) just before adding cells to the culture vessels.
5. <Optional> Poly-L-ornithine hydrobromide and laminin coating (VI-2, Steps 1 - 3) can be replaced with 0.1% gelatin type A in D-PBS (-/-) (use 0.1 ml/cm^2 vessel area; see Table 1), making sure the entire surface is covered, and incubate at RT or 37℃ ± 1℃ for 30 minutes or more.

Table 1. Coating volumes for cell culture vessels.

Cell culture vessel	0.01% poly-L-ornithine hydrobromide	10 μg/ml laminin	<Optional> 0.1% gelatin type A
6-well plate	1 ml/well	1 ml/well	1 ml/well
T25 flask	2.5 ml	2.5 ml	2.5 ml
T75 flask	7.5 ml	7.5 ml	7.5 ml

VI-3. Cell Thawing

1. Aliquot the amount of Cellartis 2i mES/iPSC Culture Medium you will use into a sterile container, and warm it to between RT and 37℃.
   [Note] Avoid prolonged heating, which causes medium inactivation.
2. Dispense 9 ml of the pre-warmed Cellartis 2i mES/iPSC Culture Medium into a 15-ml tube.
3. Thaw frozen cells in the cryovial until a small piece of ice remains.
   [Note] Thawing cells in a 1-ml vial takes 90 to 120 sec. To ensure maximum cell viability, do not let the ice completely disappear.
4. Dry the outside of the cryovial using Kimwipes, and then disinfect the vial with 70% ethanol.
5. Transfer cells from the cryovial into the tube containing 9 ml Cellartis 2i mES/iPSC Culture Medium prepared in Step 2.
6. Rinse the cryovial using 1 ml of Cellartis 2i mES/iPSC Culture Medium, and dispense this medium into the tube from Step 5.
7. Centrifuge the tube at 200*g* for 5 minutes at RT.
8. Gently aspirate the supernatant, leaving about 0.2 ml of medium in the tube. Loosen the cell pellet by gently tapping the tube.
9. Based on the cell number shown on the cryovial, add Cellartis 2i mES/iPSC Culture Medium to achieve a cell density between 5×10^5 and 1×10^6 cells/ml.
10. Count the cells and calculate the viability.
11. Aspirate D-PBS (-/-) from the cell culture vessels prepared in VI-2, Step 4 and inoculate cells at a seeding density between 4×10^4 and 6×10^4 viable cells/cm^2 (see Table 2).
12. Place the culture vessels in a 37℃, 5% CO_2 incubator.

Table 2. Reagent volumes and number of cells for cell culture vessels.

Cell culture vessel	Accutase (40 to 50 μl/cm^2)	Medium amount (0.2 ml/cm^2)	Number of cells seeded at 4×10^4 to 6×10^4 cells/cm^2
6-well plate	0.4 - 0.5 ml/well	2 ml/well	4×10^5 - 6×10^5 cells
T25 flask	1 ml	5 ml	1×10^6 - 1.5×10^6 cells
T75 flask	3 ml	15 ml	3×10^6 - 4.5×10^6 cells

VI-4. Medium Change

After seeding the cells, change the medium every day.

1. Aliquot the amount of Cellartis 2i mES/iPSC Culture Medium you will use into a sterile container, and warm it to between RT and 37℃ (see Table 2 for amounts).
2. Carefully aspirate the medium from the culture vessels and promptly add the warmed Cellartis 2i mES/iPSC Culture Medium.

<Optional>

Mouse ES and iPS cells require daily medium changes for optimal growth. However, you can occasionally culture the cells for 2 days by adding twice the volume of the medium, when the cells are seeded at 4×10^4 cells/cm^2 at step VI-3-11 or VI-5-10.

VI-5. Subculture

1. Subculture when cells cover 80 to 90% of the vessel surface.

   [Note] Do not allow cells to become overconfluent. We recommend changing the medium the day before subculturing.
2. Warm the required amount of Cellartis 2i mES/iPSC Culture Medium to between RT and 37℃.
3. Aspirate the culture medium from the culture vessels and promptly wash with the same amount of D-PBS (-/-) as the volume of medium.
4. Aspirate the D-PBS (-/-). Add accutase (cell detachment reagent) at 40 to 50 μl/cm^2 vessel area, making sure it completely covers the culture surface (see Table 2). Incubate vessels for 3 to 5 minutes at 37℃ and detach cells by gently tapping the side of the culture vessel. If microscopic observation shows insufficient detachment, incubate for another 1 to 3 minutes.

   [Note] When using a cell detachment reagent other than Accutase, please follow the manufacturer's instructions.
5. Add Cellartis 2i mES/iPSC Culture Medium at 4 times the amount of cell detachment reagent and collect it in a centrifuge tube.
6. Centrifuge the tube at 200 - 250*g* for 5 minutes at RT.
7. Slowly aspirate the supernatant, leaving about 0.2 ml of medium in the tube. Loosen the cell pellet by gently tapping the tube.
8. Based on the estimated cell number, add Cellartis 2i mES/iPSC Culture Medium to achieve a cell density between 5×10^5 and 1×10^6 cells/ml.
9. Count the cells and calculate the cell viability.
10. Aspirate the D-PBS (-/-) from the cell culture vessels prepared in VI-2, Step 4 and inoculate cells at a seeding density between 4×10^4 and 6×10^4 viable cells/cm^2 vessel area with 0.2 ml/cm^2 of Cellartis 2i mES/iPSC Culture Medium (see Table 2). The culture will become 70 - 90% confluent at 2 - 3 days after subcultured in this seeding density.
11. Incubate the vessels in a 37℃, 5% CO_2 incubator.

VI-6. Cell Freezing

1. Cryopreserve cells when cells cover 80 to 90% of the vessel surface.

   [Note] Do not allow cells to become overconfluent. We recommend changing the medium the day before cryopreservation.

2. In a sterile container, aliquot 10 times as much Cellartis 2i mES/iPSC Culture Medium as accutase cell detachment reagent. Warm the medium between RT and 37℃.
3. Aspirate the culture medium from the culture vessels and promptly wash with an equivalent volume of D-PBS (-/-) as culture medium.
4. Aspirate the D-PBS (-/-). Add accutase at 40 to 50 μl/cm^2 vessel area, making sure it completely covers the culture surface (see Table 2). Incubate for 3 to 5 minutes at 37℃ and detach cells by gently tapping the side of the culture vessel. If microscopic observation shows insufficient detachment, incubate for another 1 to 3 minutes.

   [Note] When using a cell detachment reagent other than accutase, please follow the manufacturer's instructions.

5. Add Cellartis 2i mES/iPSC Culture Medium from Step 2 at 4 times the amount of cell detachment reagent and collect it in a centrifuge tube.
6. Centrifuge the tube at 200 - 250*g* for 5 minutes at RT.
7. Slowly aspirate the supernatant, leaving about 0.2 ml of medium in the tube. Loosen the pellet by gently tapping the tube.
8. Based on the estimated cell number, add Cellartis 2i mES/iPSC Culture Medium from Step 2 to achieve a cell density between 5 x 10^5 and 1 x 10^6 cells/ml.
9. Count the cells and calculate the cell viability.
10. Centrifuge at 200 - 250*g* for 5 minutes at RT. During centrifugation, prepare the freezing container, cryopreservative, and cryovials.
11. Gently aspirate the supernatant, leaving about 0.2 ml of medium in the tube. Loosen the pellet by gently tapping the tube.
12. Add the cryopreservation solution and mix gently. As soon as the cells are evenly resuspended, promptly aliquot into the cryovials. Put the cryovials into the freezing container and store in a -80℃ deep freezer overnight.

    [Note] When freezing cells in a large number of vials, keep cells on ice after adding the cryopreservative.

13. Transfer the cryovials to liquid nitrogen storage or a -150℃ freezer.

VII. References

1. Silva J *et al.* Promotion of Reprogramming to Ground State Pluripotency by Signal Inhibition. *PLoS Biol*. (2008) **6**: e253.
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3. Nichols J *et al*. Validated germline-competent embryonic stem cell lines from non-obese diabetic mice. *Nat Med*. (2009) **15**: 814-818.
4. Wang W *et al*. Rapid and efficient reprogramming of somatic cells to induced pluripotent stem cells by retinoic acid receptor gamma and liver receptor homolog. *PNAS (USA)*. (2011) **108**(45): 18283-18288.
5. Malaver-Ortega LF *et al*. The state of the art for pluripotent stem cells derivation in domestic ungulates. *Theriogenology*. (2012) **78**(8):1749-1762.

VIII. Related Product

Cellartis® 3i mES/iPSC Culture Medium (Cat. #Y40011)

Cellartis is a registered trademark of Takara Bio Europe AB.

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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