Cat. # Y50205

For Research Use

TaKaRa

Cellartis® MSC Xeno-Free Culture Medium (w/o Phenol Red)

Product Manual

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I. Description

Mesenchymal Stem Cells (MSC) are pluripotent cells with self-renewal capacity that can differentiate into neurons, hepatocytes, pancreatic islet cells, adipocytes, chondrocytes, and osteoblasts, both *in vitro* and *in vivo*. Self-renewal capacity and pluripotency of MSC are easily lost by long-term culture and excessive passages. In order to stably maintain these cell functions, it is required to maintain their cell culture under an optimized environment.

Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red) is a xeno-free medium suitable for human MSC culture. It does not contain components, such as BSA, etc., that are derived from non-human species. Furthermore, it enables maintenance of MSC proliferation and pluripotency without plate coating reagents.*

* Coating cell culture vessels with RetroNectin® reagent or human fibronectin can further promote proliferation.

II. Contents

Cellartis MSC Xeno-Free Basal Medium (w/o Phenol Red)	475 ml (Cat. #Y50206)
Cellartis MSC Xeno-Free Supplement	25 ml (Cat. #Y50202)

III. Storage

Cellartis MSC Xeno-Free Basal Medium (w/o Phenol Red):	4℃ (Do not freeze.)
Cellartis MSC Xeno-Free Supplement:	-20℃ or below (Do not refreeze after thawing.)

IV. Precautions

1. Avoid exposure to high temperature, high humidity, ultraviolet light, and sunlight.
2. Although Cellartis MSC Xeno-Free Supplement may become slightly turbid after thawing, this precipitate does not affect performance. Mix well and use.
3. Store prepared Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red) at 4℃. Do not keep it at room temperature for a long time.
4. Use within one month after preparing Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red).
5. Before using the prepared Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red), dispense the required amount, warm this aliquot to between room temperature (RT) and 37℃. Do not warm the whole amount of medium.
6. Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red) does not contain antibiotics, and adding antibiotics is not recommended. If antibiotics must be added, the culture conditions should be optimized.
7. It is possible to culture cells with this product without using coating reagents. However, cell culture vessels precoated with RetroNectin reagent or human fibronectin can further promote proliferation. The need for a plate coating should be tested based on the experimental aim or application.

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, etc.)
- Blue ice and cooling container
- Electric pipet controller and plastic pipets
- Micropipette and sterilized tips (with filters)
- Centrifuge tubes
- Cell culture vessels

  Corning Costar Flat Bottom Cell Culture Plates:

12-well clear, tissue culture-treated plates	(Corning, Cat. #3513)
6-well clear, tissue culture-treated plates	(Corning, Cat. #3516)
25 cm^2 rectangular, canted-neck flasks with vent caps	(Corning, Cat. #430639)
75 cm^2 U-shaped, canted-neck flasks with vent caps	(Corning, Cat. #430641U)

- Cryovials
- Human Mesenchymal Stem Cells
- PBS (-/-)
- Cell detachment reagent

  Accumax (Innovative Cell Technologies, Inc., Cat. #AM105)
- Coating reagents <Optional>

  RetroNectin Recombinant Human Fibronectin Fragment (Cat. #T100A/B) or RetroNectin GMP grade (Cat. #T202)
- Cryopreservative
- Trypan blue solution
- Hemocytometer
- Ethanol for disinfection
- Kimwipes

VI. Protocol

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 MSC Xeno-Free Culture Medium (w/o Phenol Red)

1. Thaw Cellartis MSC Xeno-Free Supplement at 4℃ or RT.

   [Note] Do not leave Cellartis MSC Xeno-Free Supplement at RT for a long time after thawing. Use it quickly after thawing.

2. Add the full volume (25 ml) of thawed Cellartis MSC Xeno-Free Supplement into Cellartis MSC Xeno-Free Basal Medium (w/o Phenol Red) and mix well.

   [Note] Store prepared Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red) at 4℃ and use within one month. Do not refreeze.

VI-2. Cell Thawing

1. Aliquot the amount of Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red) you will use into a sterile container, and warm it to between RT and 37℃.

   [Note] Avoid prolonged heating, which causes medium denaturation.

2. <Optional> This step is only necessary when coating cell culture vessels. Evenly coat cell culture vessels with 10 μg/ml RetroNectin reagent (diluted in PBS) (see Table 1) and incubate at RT for at least 30 minutes.

3. Dispense 5 ml of Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red) into a 15-ml tube.

4. Thaw frozen cells until a small piece of ice remains in the cryovial.

   [Note] Thawing cells in a 1-ml vial takes 90 to 120 seconds. To ensure maximum cell survival, do not let the ice completely disappear.

5. Dry the outside of the cryovial using Kimwipes, and then disinfect the vial with ethanol.

6. Transfer cells from the cryovial into the tube containing the Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red) prepared in Step 3.

7. Rinse the cryovial using 1 ml of Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red) and dispense this medium into the tube from Step 6.

8. Centrifuge the tube at 200*g* for 5 minutes at RT.

9. Gently aspirate the supernatant, leaving about 0.2 ml of medium in the tube. Loosen the cell pellet by gently tapping the bottom of the tube.

10. Based on the cell number shown on the cryovial, add Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red) to achieve a cell density between 5 x 10^5 and 1 x 10^6 cells/ml.

11. Count the cells and calculate the survival rate.

12. Plate cells in cell culture vessels at a seeding density between 4 x 10^3 and 8 x 10^3 viable cells/cm^2 (see Table 1).

    [Notes] 1. When the cell survival rate is high, we recommend using a seeding density of 4 x 10^3 cells/cm^2.
    2. If a coating reagent was used, aspirate it before seeding.

13. Place the cultures in a 37℃, 5% CO_2 incubator.

Table 1. Reagent volumes and number of cells for various cell culture vessels.

Cell culture vessel	Coating reagent and Cell-detachment reagent	Medium amount	Number of cells seeded at 4 x 10^3 to 8 x 10^3 cells/cm^2
12-well plate	0.4 ml/well	1 ml/well	1.5 x 10^4 to 3 x 10^4 cells/well
6-well plate	1 ml/well	2 ml/well	4 x 10^4 to 8 x 10^4 cells/well
T25 flask	2.5 ml	5 ml	1 x 10^5 to 2 x 10^5 cells
T75 flask	7.5 ml	15 ml	3 x 10^5 to 6 x 10^5 cells

VI-3. Medium Change

After seeding cells, change the medium every two to three days, depending on the growth rate (see Table 2).

1. Aliquot the amount of Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red) you will use into a sterile container, and warm it to between RT and 37℃.
2. Carefully aspirate the medium from the culture vessels and promptly add fresh Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red) (see Table 1 for amounts).

Table 2. Culturing schedule based on growth rate.

Growth rate	Fast	Medium	Slow
Day 0	Thawing/seeding or subculture		
Day 1			
Day 2	Medium change		Medium change
Day 3	Subculture	Medium change	
Day 4		Subculture	Medium change
Day 5			Subculture

VI-4. Cell Subculture

1. Subculture when cells reach 70 to 80% confluency.

   [Note] Be careful to not become confluent culture, because confluent cells may be detached as cell sheet after treating of cell detachment reagent and not become single cells. Subculture the cells at suitable confluency by observation once or twice in a day. We recommend changing the medium the day before subculturing.

2. Warm the required amount of Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red) to between RT and 37℃.

3. <Optional> This step is only necessary when coating cell culture vessels. Evenly coat cell culture vessels with 10 μg/ml RetroNectin reagent (diluted in PBS) (see Table 1) and incubate at RT for at least 30 minutes.

4. Aspirate the culture medium from the culture vessels and promptly wash with the same amount of PBS as the volume of medium that was aspirated.

5. Aspirate the PBS. Add Accumax (cell detachment reagent) at 100 μl/cm^2, making sure it completely coats the culture surface (see Table 1). Incubate vessels for 5 to 7 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 Accumax, please follow the manufacturer's instructions.

6. Collect cells in a centrifuge tube. Rinse culture vessels using the same amount of Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red) as cell detachment reagent added, and collect it in the same centrifuge tube. Dilute the cell suspension with Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red), using 5 to 10 times the amount of cell detachment reagent added.

7. Centrifuge the tube at 200*g* for 5 minutes at RT.

8. Slowly aspirate the supernatant, leaving about 0.2 ml of medium in the tube. Loosen the pellet by gently tapping the bottom of the tube.

9. Based on the estimated cell number, add Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red) to achieve a cell density between 5 x 10^5 and 1 x 10^6 cells/ml.

10. Count the cells and calculate the survival rate.

11. Plate cells in cell culture vessels at a seeding density between 4 x 10^3 and 8 x 10^3 viable cells/cm^2 (see Table 1).

    [Note] If a coating reagent was used, aspirate it before seeding.

12. Place the cultures in a 37℃, 5% CO_2 incubator.

VI-5. Cell Freezing

1. Cryopreserve when cells reach 70 to 80% confluency.

   [Note] Be careful to not become confluent culture, because confluent cells may be detached as cell sheet after treating of cell detachment reagent and not become single cells. Subculture the cells at suitable confluency by observation once or twice in a day. We recommend changing the medium the day before cryopreservation.

2. In a sterile container, aliquot 10 times as much Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red) as cell detachment reagent needed. Warm the medium between RT and 37℃.

3. Aspirate the culture medium from the culture vessels and promptly wash with an equivalent volume of PBS as culture medium removed.

4. Aspirate the PBS. Add Accumax at 100 μl/cm^2, making sure it completely coats the culture surface (see Table 1). Incubate vessels for 5 to 7 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 Accumax, please follow the manufacturer's instructions.

5. Collect cells in a centrifuge tube. Rinse culture vessels with the same amount of medium as cell detachment reagent used. Add this to the same centrifuge tube.

6. Count the cells and calculate the survival rate.

7. Calculate the volume of cryopreservative based on the number of cells.

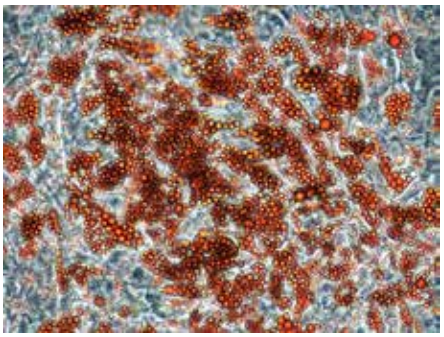
8. Centrifuge at 200*g* for 5 minutes at RT. During centrifugation, prepare the freezing container, cryopreservative, and cryovials.

9. Gently aspirate the supernatant, leaving about 0.2 ml of medium in the tube. Loosen the pellet by gently tapping the bottom of the tube.

10. Add the cryopreservative and mix gently. As soon as the cells are evenly resuspended, promptly aliquot into the cryovials. Put the cryovials into the freezing container and place in a -80℃ deep freezer overnight.

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

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



(Left) Human Mesenchymal Stem Cells from Bone Marrow cultured with Cellartis MSC Xeno-Free Culture Medium

(Right) Oil Red O staining shows directed differentiation into adipocytes by MSC Adipogenic Differentiation Medium 2 (PromoCell, Cat. #C-28016) after culturing Human Mesenchymal Stem Cells from Bone Marrow with Cellartis MSC Xeno-Free Culture Medium.

VII. Related Products

[Medium]
Cellartis® MSC Xeno-Fre Culture Medium (Cat. #Y50200)

[Coating reagents]
RetroNectin® Recombinant Human Fibronectin Fragment (Cat. #T100A/B)
RetroNectin® GMP grade (Cat. #T202)