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iCell® Mesenchymal Stem Cells

User's Guide

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CDI does not in any way guarantee or represent that you will obtain satisfactory results from using iCell Mesenchymal Stem Cells as described herein. You assume all risk in connection with your use of iCell Mesenchymal Stem Cells.

Conditions of Use

iCell Mesenchymal Stem Cells are for life science research use only and subject to the use restrictions contained in Appendix A. You are responsible for understanding and performing the protocols described within this guide. CDI does not guarantee any results you may achieve. These protocols are provided as CDI’s recommendations based on its use and experience with iCell Mesenchymal Stem Cells.

Origin

iCell Mesenchymal Stem Cells are manufactured in the United States of America.

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Before You Begin

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire iCell® Mesenchymal Stem Cells User's Guide before handling or using iCell Mesenchymal Stem Cells.
- iCell Mesenchymal Stem Cells are for life science research use only. See Appendix A for more information and other restrictions.
- A Safety Data Sheet (SDS) for dimethyl sulfoxide (DMSO), in which iCell Mesenchymal Stem Cells are frozen, is available online at www.cellulardynamics.com/lit/ or on request from Cellular Dynamics International. Only technically qualified individuals experienced in handling DMSO and human biological materials should access, use, or handle iCell Mesenchymal Stem Cells.

Notes

Chapter 1. Introduction

Cellular Dynamics International's (CDI) iCell Mesenchymal Stem Cells are a highly pure population of human mesenchymal stem cells derived from induced pluripotent stem (iPS) cells using CDI's proprietary differentiation protocols. iCell Mesenchymal Stem Cells exhibit characteristic gene and protein expression and multilineage differentiation potential (i.e. adipocyte, chondrocyte, and osteocyte). These cells provide a reliable source of human mesenchymal stem cells suitable for use in targeted drug discovery, toxicity testing, and other life science research.

When thawed and plated according to this User's Guide, iCell Mesenchymal Stem Cells can be maintained in culture for up to 5 passages. These cells are suitable for an array of assays including but not limited to flow cytometry, qPCR, and immunomodulatory assays.

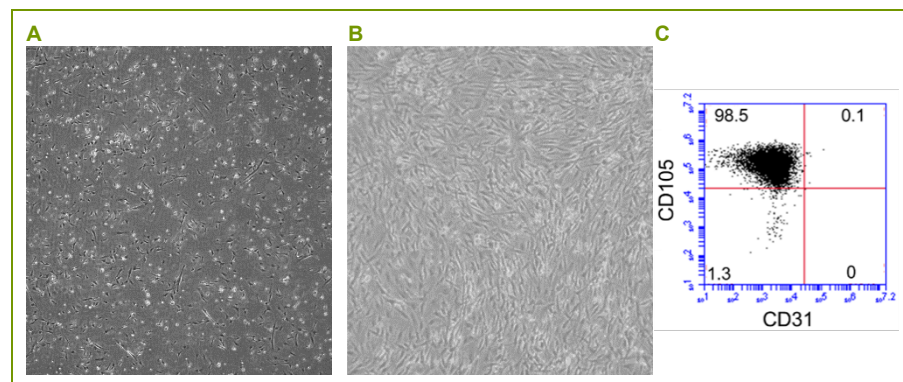


Figure 1: iCell Mesenchymal Stem Cells Represent a Highly Pure Population of Human Mesenchymal Stem Cells

These images show iCell Mesenchymal Stem Cells at (A) day 1 and (B) day 5 post-plating. iCell Mesenchymal Stem Cells form a confluent monolayer in culture in approximately 5 days post-plating and (C) show the appropriate human mesenchymal stem cell markers as demonstrated by flow cytometry.

Components Supplied by Cellular Dynamics

Notes

Item	Catalog Number
iCell Mesenchymal Stem Cells Kit, 01279	R1098
<ul style="list-style-type: none"> • iCell Mesenchymal Stem Cells, 01279^{1, 2} • iCell Mesenchymal Stem Cells User's Guide² 	<ul style="list-style-type: none"> • C1043 ($\geq 1.0 \times 10^6$ viable cells) • X1017
Certificate of Testing ³	
Certificate of Origin If required for shipping purposes	
<p>1 This product was formerly known by this name and catalog number: iCell Mesenchymal Stem Cells Prototype (Cat. No. MSC-301-010-001-PT)</p> <p>Note: You may receive product labeled with its former name and catalog number until current stock is depleted. There were no changes in the manufacture of the cells.</p> <p>2 Safety Data Sheet and User's Guide available online: www.cellulardynamics.com/lit/</p> <p>3 Available online: www.cellulardynamics.com/cot/</p>	

Required Equipment and Consumables

Note: As required for the intended use, see the following iCell Mesenchymal Stem Cells Application Protocols for assay-specific equipment and consumables before thawing cells:

- Modeling Adipocyte Differentiation
- Modeling Chondrocyte Differentiation
- Modeling Osteocyte Differentiation

These Application Protocols are available online: www.cellulardynamics.com/lit/

Item	Vendor	Catalog Number
Equipment		
37°C Water Bath	Multiple Vendors	
Biological Safety Cabinet with UV Lamp	Multiple Vendors	
Cell Culture Incubator	Multiple Vendors	
Hemocytometer or Automated Cell Counter ¹	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
1-Thioglycerol (MTG)	Sigma-Aldrich	M6145
6-well Flat-bottom Plate, TC-treated, Falcon ²	STEMCELL Technologies	38016
Ascorbic Acid	Multiple Vendors	
B-27 Supplement, Minus Vitamin A	Thermo Fisher Scientific	12587
bFGF ³	Multiple Vendors	
Bovine Serum Albumin (BSA)	Sigma-Aldrich	A1470
Conical Tubes, 15 ml, Falcon (Centrifuge Tubes) ²	STEMCELL Technologies	38009
Dulbecco's Phosphate Buffered Saline without Ca ²⁺ and Mg ²⁺ (D-PBS) ²	STEMCELL Technologies	37350
Fetal Bovine Serum (FBS)	Multiple Vendors	
GlutaMAX Supplement	Thermo Fisher Scientific	35050
Ham's F12 Medium	Corning	10-080-CV
Iscove's Modified Dulbecco's Medium (IMDM) ²	STEMCELL Technologies	36150

Notes

Item	Vendor	Catalog Number
N-2 Supplement	Thermo Fisher Scientific	17505
PDGF-BB	Multiple Vendors	
Penicillin/Streptomycin	Thermo Fisher Scientific	15140
PES Filter Unit, 0.2 µm, 500 ml	Multiple Vendors	
Tissue Culture-treated Dishes, 100 mm ²	STEMCELL Technologies	38046
Trypan Blue ²	STEMCELL Technologies	07050
TrypLE Select Enzyme, No Phenol Red (TrypLE)	Thermo Fisher Scientific	12563

- 1 Ensure the automated cell counter is appropriately calibrated before use.
- 2 Similar products are available from multiple vendors.
- 3 This User's Guide provides instructions for using bFGF from zebrafish in the Maintenance Medium. However, other commercially available sources for bFGF, which have not been evaluated by CDI, may work.

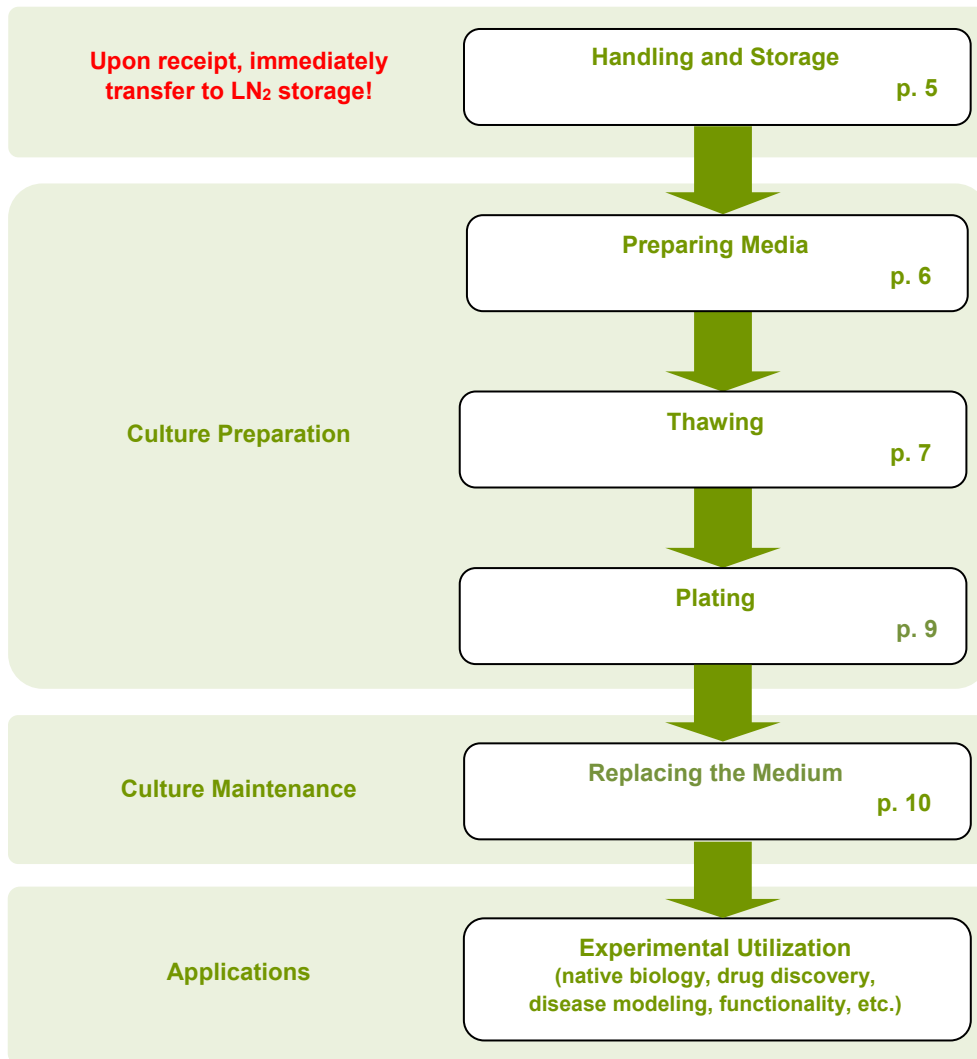
Technical Support, Knowledge Base, and Training

CDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. Our web-based Knowledge Base provides solutions for iCell related questions about plating and media, cell culture, general assay methods, and more. In addition, in-lab training may be available upon request.

Telephone	(877) 320-6688 (US toll-free) / (608) 310-5100 x5 Monday - Friday, 8:30 am - 5:00 pm US Central Time
Fax	(608) 310-5101
Email	support@cellulardynamics.com
Knowledge Base	www.cellulardynamics.com/knowledgebase/

Workflow Diagram

Notes



Chapter 2. Handling and Storage

iCell Mesenchymal Stem Cells are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the cryobox containing iCell Mesenchymal Stem Cells to the vapor phase of liquid nitrogen storage dewar. CDI strongly recommends transferring the entire cryobox into the storage rack to avoid transferring individual vials.



It is critical to maintain cryopreserved iCell Mesenchymal Stem Cells at a stable temperature. Minimize exposure of cryopreserved iCell Mesenchymal Stem Cells to ambient temperature when transferring vials to liquid nitrogen storage.

Chapter 3. Preparing Media

Notes

Note: *iCell Mesenchymal Stem Cells will plate and function optimally on tissue culture treated vessels.*

Prepare and store Plating Medium and Maintenance Medium for iCell Mesenchymal Stem Cells as follows:

Plating Medium Component	Amount (ml)	Final Concentration
FBS	5	10%
IMDM	45	90%

- 1 Filter Plating Medium using a 0.2 μ m PES filter unit.
- 2 Store Plating Medium at 4°C for up to 2 weeks.

Maintenance Medium Component	Amount (ml)	Final Concentration
Ascorbic Acid, 20 mg/ml	2.5	50 μ g/ml
B-27 Supplement, Minus Vitamin A, 50X	10	1%
bFGF, 1 mg/ml	0.05	50 ng/ml
BSA, 10% in D-PBS	5	0.05%
GlutaMAX Supplement	10	1%
Ham's F-12 Medium	250	25%
IMDM	750	75%
MTG, 11.5 M	0.04	450 μ M
N-2 Supplement, 100X	5	0.5%
PDGF-BB, 100 μ g/ml	0.5	50 ng/ml
Penicillin/Streptomycin	10	1%

- 1 Prepare 10% BSA in D-PBS. Filter 10% BSA using a 0.2 μ m PES filter unit. Store remaining 10% BSA at 4°C for up to 1 month.
- 2 Filter Maintenance Medium using a 0.2 μ m PES filter unit.
- 3 Store Maintenance Medium at 4°C for up to 2 weeks.

Chapter 4. Thawing iCell Mesenchymal Stem Cells

Maintain iCell Mesenchymal Stem Cells in liquid nitrogen until immediately before thawing to ensure maximal performance of the cells. Complete the following steps of the thawing protocol in a time-efficient manner to facilitate optimal iCell Mesenchymal Stem Cells viability and performance.

1. Equilibrate Plating Medium and Maintenance Medium at room temperature before thawing iCell Mesenchymal Stem Cells.
2. Remove the iCell Mesenchymal Stem Cells cryovial from the liquid nitrogen storage tank.

Note: *If necessary, place cryovials on dry ice for up to 10 minutes before thawing.*

3. Immerse the cryovial in a 37°C water bath and gently swirl (avoid submerging the cap) until the cell suspension is fully thawed. Use of a floating microcentrifuge tube rack is recommended.
4. When the cell suspension is fully thawed, immediately remove the cryovial from the water bath, spray with 70% ethanol, and place in a biological safety cabinet.
5. Gently transfer the iCell Mesenchymal Stem Cells cryovial contents to a sterile 15 ml centrifuge tube using a 1 ml pipettor.



Avoid repeated pipetting of the thawed iCell Mesenchymal Stem Cells suspension.

6. Rinse the empty iCell Mesenchymal Stem Cells cryovial with 1 ml of room temperature Plating Medium to recover residual cells from the cryovial. Transfer the 1 ml of Plating Medium rinse drop-wise to the 15 ml centrifuge tube containing the iCell Mesenchymal Stem Cells suspension. Gently swirl the tube while adding the medium to mix the solution completely and minimize the osmotic shock on the thawed cells.



Drop-wise addition of Plating Medium to the cell suspension is critical to minimize osmotic shock and ensure maximum viability and subsequent attachment of the cells.

7. Slowly add 8 ml of Plating Medium to the 15 ml centrifuge tube. Gently swirl the tube while adding the Plating Medium.



It is critical to add the 8 ml of Plating Medium slowly to ensure maximum viability and attachment of the cells once plated.

8. Gently mix the contents of the 15 ml centrifuge tube by pipetting the cell suspension once. Avoid repeated pipetting, vigorous shaking, or vortexing of the cell suspension.

9. Centrifuge the cell suspension at 400 x g for 5 minutes at room temperature.
10. Carefully aspirate the supernatant, taking care not to disturb the cell pellet.
11. Slowly add 5 ml of Maintenance Medium to resuspend the cell pellet.

Note: Thaw no more than 3 vials of iCell Mesenchymal Stem Cells at one time. Once thawed, combine the contents of the cryovials after adding the rinse and final volume of Plating Medium. For example, if pooling 3 cryovials, add each 1 ml of rinse and 8 ml of additional Plating Medium. Once thawed and diluted to the desired density, cells can be pooled together for plating.

Notes

Chapter 5. Plating iCell Mesenchymal Stem Cells

The following procedure describes how to plate iCell Mesenchymal Stem Cells at 35,000 viable cells/cm² into a 6-well cell culture plate. Scale volumes appropriately for other cell culture vessel formats.

1. Invert the thawed iCell Mesenchymal Stem Cells suspension 2 - 3 times to ensure an even cell distribution before performing the cell count.
2. Remove a sample of cells to confirm the viability of the cells using a hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter.
3. Dilute the cell suspension in room temperature Maintenance Medium to 1.68 x 10⁵ cells/ml).

Culture Vessel	Surface Area (cm ²)	Maintenance Medium (ml)	Cell Number (3.5 x 10 ⁴ cells/cm ²)
6-well Cell Culture Plate	9.6	2	3.36 x 10 ⁵
10 cm Cell Culture Dish	57	10	1.995 x 10 ⁶

Table 1. Summary of Recommended Volumes and Measures

All volumes and measures are per well, if applicable.

4. Invert the cell suspension 2 - 3 times. Immediately dispense 2 ml/well of cell suspension into the cell culture vessel (~336,000 viable cells/well).
5. Culture iCell Mesenchymal Stem Cells in a cell culture incubator at 37°C, 5% CO₂.

Chapter 6. Maintaining iCell Mesenchymal Stem Cells

iCell Mesenchymal Stem Cells are cryopreserved at high purity. The cells retain their high purity when maintained and cultured as recommended.

1. Equilibrate Plating Medium and Maintenance Medium at room temperature before use.
2. 48 hours post-plating iCell Mesenchymal Stem Cells, aspirate the spent medium and replace with the appropriate volume of fresh Maintenance Medium.
3. Replace the Maintenance Medium every other day.
4. Culture iCell Mesenchymal Stem Cells in a cell culture incubator at 37°C, 5% CO₂.
5. Passage iCell Mesenchymal Stem Cells at 80% - 90% confluency. The following instructions apply to one well of a 6-well cell culture plate. Scale volumes appropriately for other vessel formats.

Note: *iCell Mesenchymal Stem Cells will plate and function optimally on tissue culture treated vessels.*

- a. Aspirate the spent medium from the 80% - 90% confluent cell culture.
- b. Add 1 ml/well of TrypLE.
- c. Incubate at 37°C until cells detach from the plate (approximately 5 - 7 minutes).
- d. Tap the vessel gently to dislodge cells from the plate. Gently pipette up and down once to dislodge cells. Transfer the cell suspension to a 15 ml centrifuge tube. Microscopically check the plate to confirm that cells have been removed.



Do not remove cells from the plate by scraping. If cells are not harvested according to these instructions, there may be a loss of viability, poor recovery, or loss of cell surface markers.

- e. Rinse each well with 2 ml of Plating Medium. Transfer the 2 ml rinse from the well to the 15 ml centrifuge tube containing the iCell Mesenchymal Stem Cells suspension to quench the TrypLE.
- f. Resuspend cells by gently pipetting. Remove a sample of cells to perform a cell count using a hemocytometer or an automated cell counter.
- g. Centrifuge the cell suspension at 400 x g for 5 minutes.
- h. Carefully aspirate the supernatant, taking care not to disturb the cell pellet.
- i. Resuspend cells in the appropriate volume of Maintenance Medium to plate iCell Mesenchymal Stem Cells at a density of 35,000 cells/cm² (i.e. 336,000 cells/well in 2 ml of Maintenance Medium for a 6-well cell culture plate). Suggested cell numbers for different vessel formats are provided in Table 1 on page 9.

Notes

6. Add iCell Mesenchymal Stem Cells into the culture vessel.

Note: *iCell Mesenchymal Stem Cells can be maintained by routine splitting for up to 5 passages. To avoid a poor quality cell culture, do not allow cells to become over-confluent at any time. It is recommended to passage at 80% - 90% confluency.*

Appendix A. Intellectual Property Rights, Use Restrictions, and Limited License

A. OWNERSHIP. The Products are covered by pending patents and patents: cellulardynamics.com/about-us/patents/. Customer has a limited license to use the Products for internal research purposes for the sole benefit of the Customer, subject to the use restrictions included in subsection B of this Appendix A. Customer acknowledges and agrees that the receipt or purchase of the Products by Customer shall not be construed as a transfer of any title or the grant of any rights in or to the intellectual property embodied in the Products owned or licensed by Cellular Dynamics. In particular, no right or license to make, have made, offer to sell, or sell the Products, to modify or reproduce the Product or any part thereof, or to use the Products in combination with any other product(s), except product(s) provided or expressly licensed to Customer by Cellular Dynamics for such use, is implied or conveyed by the sale or transfer of Products to Customer.

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C. DATA. Customer agrees that if described on Customer's product quotation from Cellular Dynamics it will provide data and information as described therein to Cellular Dynamics regarding Customer's use of the Products.

Appendix B. Product Provided "AS IS"

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C. Customer will be solely responsible for (i) Customer's use of the Products for a purpose or in a manner other than that for which they were designed or that is permitted or in breach of the Use Restrictions above; (ii) Customer's failure to follow this User's Guide for the use,

Notes

storage, and handling of the Products however such failure is caused; (iii) Customer's failure to comply with any of the provisions of Appendix A above; and (iv) any abuse, other misuse or neglect of the Products by Customer or any damage or loss of the Products by events or occurrences beyond a person's (e.g., Cellular Dynamics') control including without limitation, accident, fire, vandalism and natural disasters (acts of God).

D. Customer acknowledges and agrees that Cellular Dynamics may fill Customer's order with any number of units of Products. Such units may be more units than Customer ordered. Customer will not be charged extra for any adjustments made by Cellular Dynamics. Because the number of cells in a unit may vary from lot to lot, Cellular Dynamics reserves the right to fill the order with that number of units which is sufficient to fill Customer's order.

Appendix C. Limited Liability

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