Cellular Dynamics international

iCell® Skeletal Myoblasts Prototype User's Guide

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iCell Skeletal Myoblasts are for life science research use only and subject to the use restrictions as contained in Appendix A. You are responsible for understanding and performing the protocols described within. 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 Skeletal Myoblasts.

Origin

iCell Skeletal Myoblasts are manufactured in the United States of America.

Copyright Notice

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Before You Begin

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire iCell® Skeletal Myoblasts Prototype User's Guide before handling or using iCell Skeletal Myoblasts.
- iCell Skeletal Myoblasts 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 Skeletal Myoblasts 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 Skeletal Myoblasts.

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Chapter 1. Introduction

Cellular Dynamics International's (CDI) iCell Skeletal Myoblasts are a highly pure population of human skeletal myoblasts derived from induced pluripotent stem (iPS) cells. Upon thaw and culture in specific serum-free medium, iCell Skeletal Myoblasts fuse to form myotubes, thus providing a reliable source of this material suitable for use in targeted drug discovery, toxicity testing, and other life science research.

Figure 1: iCell Skeletal Myoblasts Represent a Highly Pure Population of Human Myoblasts that Form Myotubes in Culture
These images show iCell Skeletal Myoblasts at days 1 and 6 post-plating. iCell Skeletal Myoblasts form myotubes in culture within 6 days post-plating as demonstrated by immunocytochemistry: Troponin T (green) and Hoechst (blue).

Components Supplied by Cellular Dynamics

Item	Catalog Number
iCell Skeletal Myoblasts Prototype[1]	SKM-301-020-001-PT
iCell Skeletal Myoblasts Prototype User's Guide[1]	
Certificate of Testing[2]	
Certificate of Origin If required for shipping purposes	

1 Safety Data Sheet and User's Guide available online at www.cellulardynamics.com/lit/

2 Available by emailing support@cellulardynamics.com or calling (877) 320-6688 (US toll-free) or (608) 310-5100

Required Equipment and Consumables

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	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
15 ml and 50 ml Centrifuge Tubes	Multiple Vendors	
96-well Cell Culture Plates	Multiple Vendors	
B27 Supplement, 50X	Life Technologies	17504-044
CHIR99021, 10 mg	StemGent	04-00040-10
DMEM, Low Glucose	HyClone	SH30021.02
DMEM/F12	Life Technologies	11330-032
Growth Factor Reduced Corning Matrigel Matrix (Matrigel)	Corning	354230
PES Filter Unit, 0.2 µm, 500 ml	Multiple Vendors	
Pipettes	Multiple Vendors	

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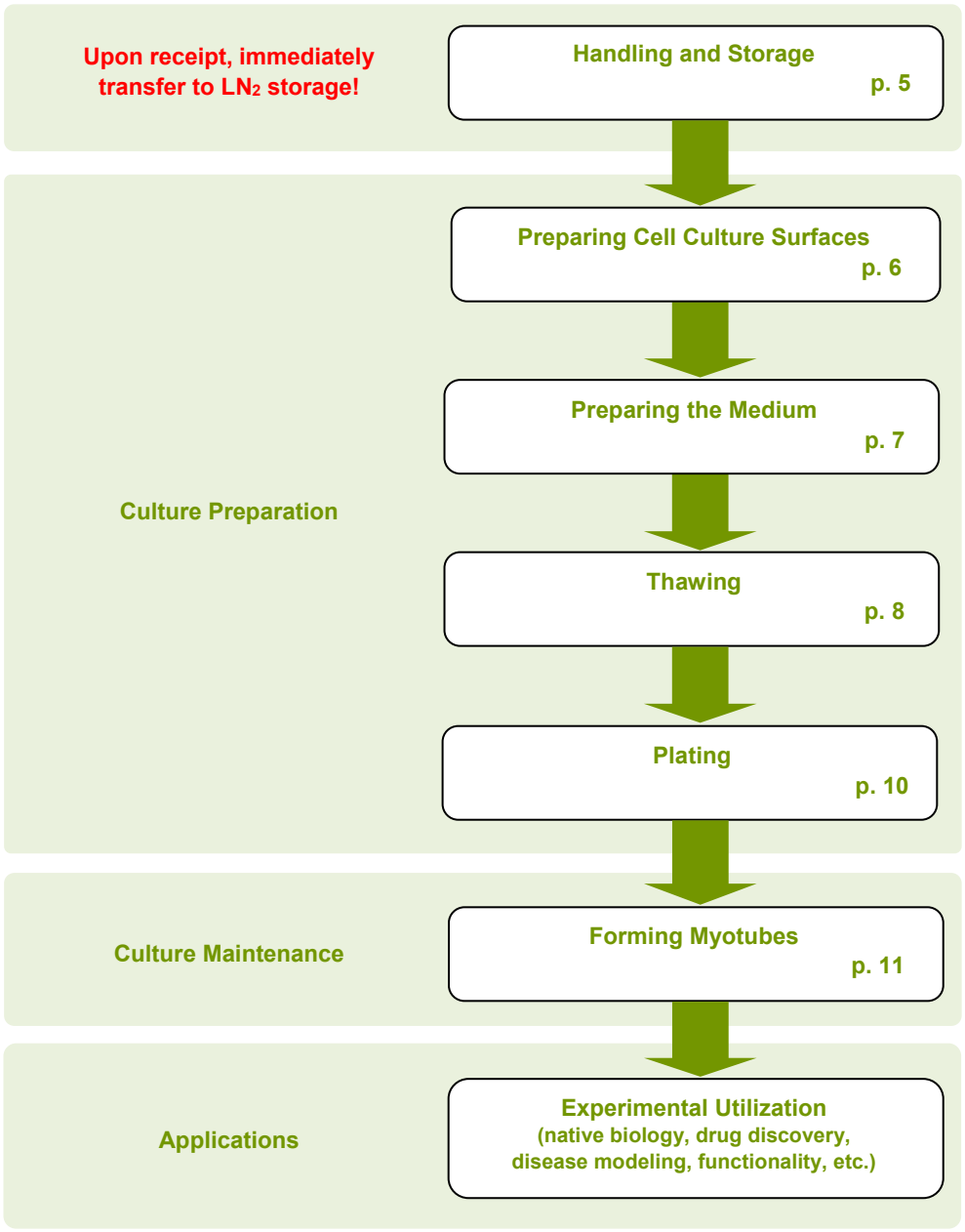
Technical Support and Training

CDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. 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

Workflow Diagram



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Chapter 2. Handling and Storage

iCell Skeletal Myoblasts are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the cryobox containing iCell Skeletal Myoblasts to the vapor phase of a 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 Skeletal Myoblasts at a stable temperature. Minimize exposure of cryopreserved iCell Skeletal Myoblasts to ambient temperature when transferring vials to liquid nitrogen storage.

Chapter 3. Preparing Cell Culture Surfaces

Notes

iCell Skeletal Myoblasts will plate and function on cell culture vessels pre-coated with Matrigel. The following procedure details coating 96-well cell culture plates. Scale volumes appropriately for other vessel formats.

1. Dilute the Matrigel in ice-cold DMEM/F12 to a final concentration of 0.083 mg/ml.
2. Immediately add 100 µl/well of the cell culture vessel(s).
3. Incubate the vessel(s) at room temperature for 1 hour or at 4°C overnight before plating iCell Skeletal Myoblasts.

   Note: *Plates coated with Matrigel can be stored at 4°C for up to 1 week. Equilibrate the plates in a 37°C cell culture incubator before use.*

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Chapter 4. Preparing the Medium

iCell Skeletal Myoblasts Maintenance Medium (Maintenance Medium) is comprised of DMEM (low glucose), B27 supplement, and CHIR99021. The Maintenance Medium is serum-free and antibiotic-free.

1. Reconstitute the CHIR99021 in DMSO at 20 mM according to the manufacturer's instructions. Aliquot and store at -20°C for future use.
2. Thaw the B27 supplement at 4°C overnight (or in a 37°C water bath just until thawed).
3. Prepare the Maintenance Medium by adding the following components:

Component	Final Concentration
DMEM, Low Glucose	98%
B27 Supplement, 50X	1X
CHIR99021, 20 mM	2 µM

Table 1: Components of Maintenance Medium

4. Filter the Maintenance Medium using a 500 ml, 0.2 µm PES filter unit.
5. Store the Maintenance Medium at 4°C, protected from light, for up to 1 week.

Chapter 5. Thawing iCell Skeletal Myoblasts

Notes

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

Note: *Thaw no more than 3 vials of iCell Skeletal Myoblasts at one time.*

1. Equilibrate the Maintenance Medium at room temperature for 2 - 4 hours before thawing iCell Skeletal Myoblasts.
2. Remove the iCell Skeletal Myoblasts 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 for 3 minutes (avoid submerging the cap) holding the tube stationary (no swirling). Use of a floating microcentrifuge tube rack is recommended.
4. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place into the biological safety cabinet.
5. Gently transfer the iCell Skeletal Myoblasts cryovial contents to a sterile 50 ml centrifuge tube using a 1 ml pipettor.

   Note: *Use of a 50 ml centrifuge tube facilitates suitable mixing to minimize osmotic shock and increase myoblasts viability.*

   

   Avoid repeated pipetting of the thawed iCell Skeletal Myoblasts cell suspension.
6. Rinse the empty iCell Skeletal Myoblasts cryovial with 1 ml of room temperature Maintenance Medium to recover any residual cells from the cryovial. Transfer the 1 ml of Maintenance Medium rinse from the cryovial drop-wise (~1 drop/second) to the 50 ml centrifuge tube containing the iCell Skeletal Myoblasts cell 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 Maintenance Medium to the cell suspension is critical to minimize osmotic shock and ensure maximum viability and subsequent attachment of the cells to the plating substrate.
7. Slowly add 8 ml of room temperature Maintenance Medium to the 50 ml centrifuge tube (~1 - 2 drops/second). Gently swirl the centrifuge tube while adding the medium.

   

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

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8. Continue to gently mix the contents of the 50 ml centrifuge tube by swirling or inverting 2 - 3 times. Gentle mixing is critical to ensure maximum viability. Avoid vigorous shaking or vortexing of the cell suspension.

   Note: *iCell Skeletal Myoblasts can be concentrated post-thawing. Transfer the cell suspension to a 15 ml centrifuge tube and centrifuge at 300 x g for 5 minutes. Aspirate the supernatant, leaving 1 ml in the centrifuge tube, and resuspend the cell pellet in Maintenance Medium to the desired concentration.*

Chapter 6. Plating iCell Skeletal Myoblasts

Notes

The recommended plating density for iCell Skeletal Myoblasts is ~2.6 - 3.2 x 10^5 cells/cm^2 (0.8 - 1.0 x 10^5 viable cells/well of a 96-well cell culture plate).

1. Remove a sample of cells to perform a cell count using a hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter.
2. Dilute the cell suspension using room temperature Maintenance Medium to obtain a desired cell plating density.
3. Aspirate the Matrigel from the pre-coated cell culture vessel(s) and immediately dispense the cell suspension.
4. Culture iCell Skeletal Myoblasts in a cell culture incubator at 37°C, 5% CO_2.

Expected Cell Density

~2.6 - 3.2 x 10^5 cells/cm^2 is the recommended starting density of iCell Skeletal Myoblasts for myotube formation. However, the optimal density of iCell Skeletal Myoblasts per unit of surface area can be assay dependent and must be determined empirically based on the intended use. The following table provides the desired cell number and plating volume for several common culture vessels.

Culture Vessel	Surface Area (cm^2)	Plating Volume (ml)	Cell Number (~2.6 - 3.2 x 10^5 cells/cm^2)
6-well Cell Culture Plate	9.6	3	2.4 - 3 x 10^6
24-well Cell Culture Plate	1.9	0.6	4.9 - 6 x 10^5
96-well Cell Culture Plate	0.32	0.2	0.8 - 1 x 10^5

Table 2: Summary of Recommended Volumes and Measures
*All volumes and measures are **per well**.*

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Chapter 7. Forming Myotubes from iCell Skeletal Myoblasts

1. Immediately before use, equilibrate the Maintenance Medium in a 37°C water bath.
2. 24 hours post-plating iCell Skeletal Myoblasts, gently remove the non-adherent cells and debris by pipetting the spent medium up and down twice, each time carefully dispensing the medium against the side of the well.
3. Aspirate the spent medium and replace (100% exchange) with the appropriate volume of 37°C Maintenance Medium. Recommend volumes are as follows:
   - **6-well cell culture plate:** 2 ml/well
   - **24-well cell culture plate:** 0.6 ml/well
   - **96-well cell culture plate:** 100 µl/well
4. Replace the spent medium every 2 - 3 days.

   ***Note**: Myotubes form by day 6 post-plating and can be maintained for at least 8 additional days.*
5. Culture the myotubes in a cell culture incubator at 37°C, 5% CO_2.

Appendices

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

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