Cellular Dynamics international

iCell® Macrophages Prototype 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 Macrophages as described herein. You assume all risk in connection with your use of iCell Macrophages.

Conditions of Use

iCell Macrophages 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 Macrophages.

Origin

iCell Macrophages are manufactured in the United States of America.

Copyright Notice

Revision History

Document #DX21837
Version 0.2: August 2014
Version 0.1: July 2014

Table of Contents

Before You Begin

Notes

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire iCell® Macrophages Prototype User's Guide before handling or using iCell Macrophages.
- iCell Macrophages 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 Macrophages are frozen, is available online at www.cellulardynamics.com/sds/ 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 Macrophages.

Notes

Chapter 1. Introduction

Cellular Dynamics International's (CDI) iCell Macrophages are highly purified, human macrophages derived from induced pluripotent stem (iPS) cells using CDI's proprietary differentiation and purification protocols. iCell Macrophages exhibit expected physiological characteristics and responses. iCell Macrophages are capable of phagocytotic uptake of substrates, such as ovalbumin, and release IL6 and TNFα in response to LPS stimulation. Thus, these cells provide a reliable source of human macrophages suitable for use in targeted drug discovery, toxicity testing, and other life science research.

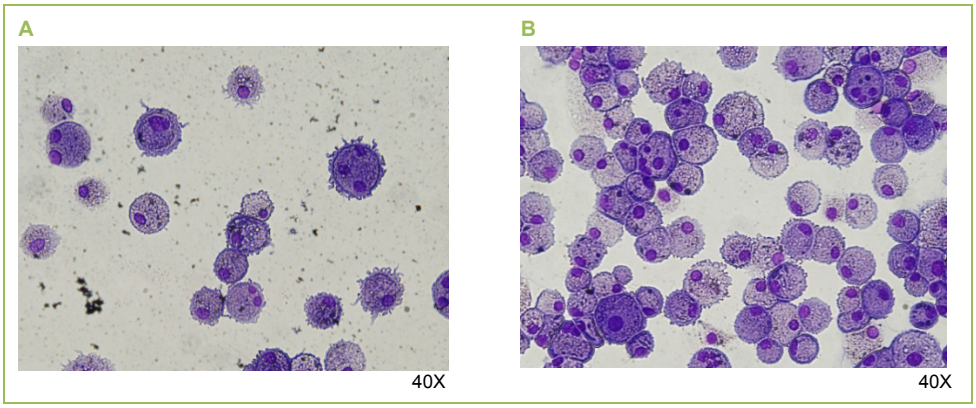

Figure 1: iCell Macrophages Represent a Highly Pure Population of Human Macrophages
These images show iCell Macrophages cytospins stained with Wright stain: (A) 10,000 cells per spot and (B) 50,000 cells per spot.

Components Supplied by Cellular Dynamics

Item	Catalog Number
iCell Macrophages Prototype[1]	MAC-301-010-001-PT
iCell Macrophages Prototype User's Guide[2]	
Certificate of Testing[2]	
Certificate of Origin If required for shipping purposes	

1 Safety Data Sheet available online at www.cellulardynamics.com/sds/

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	
Nunclon Delta Tissue Culture Vessel (Cell Culture Plate)*	Nunc	140675 (6-well) 150628 (12-well) 142475 (24-well) 167008 (96-well)
Phase Contrast Microscope	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
50 ml Centrifuge Tubes	Multiple Vendors	
ExCyte	Millipore	81-129-1
FBS	Hyclone	SH30396.03
Glutamax	Life Technologies	35050
IL1β	Multiple Vendors	
IMDM	Life Technologies	12440
MCSF	Multiple Vendors	
PES Filter Unit, 0.2 µm	Multiple Vendors	
Pipettes	Multiple Vendors	
SFEM	Stem Cell Technologies	09650
Trypan Blue	Gibco	15250

* Order the format of cell culture plate required for your experiment.

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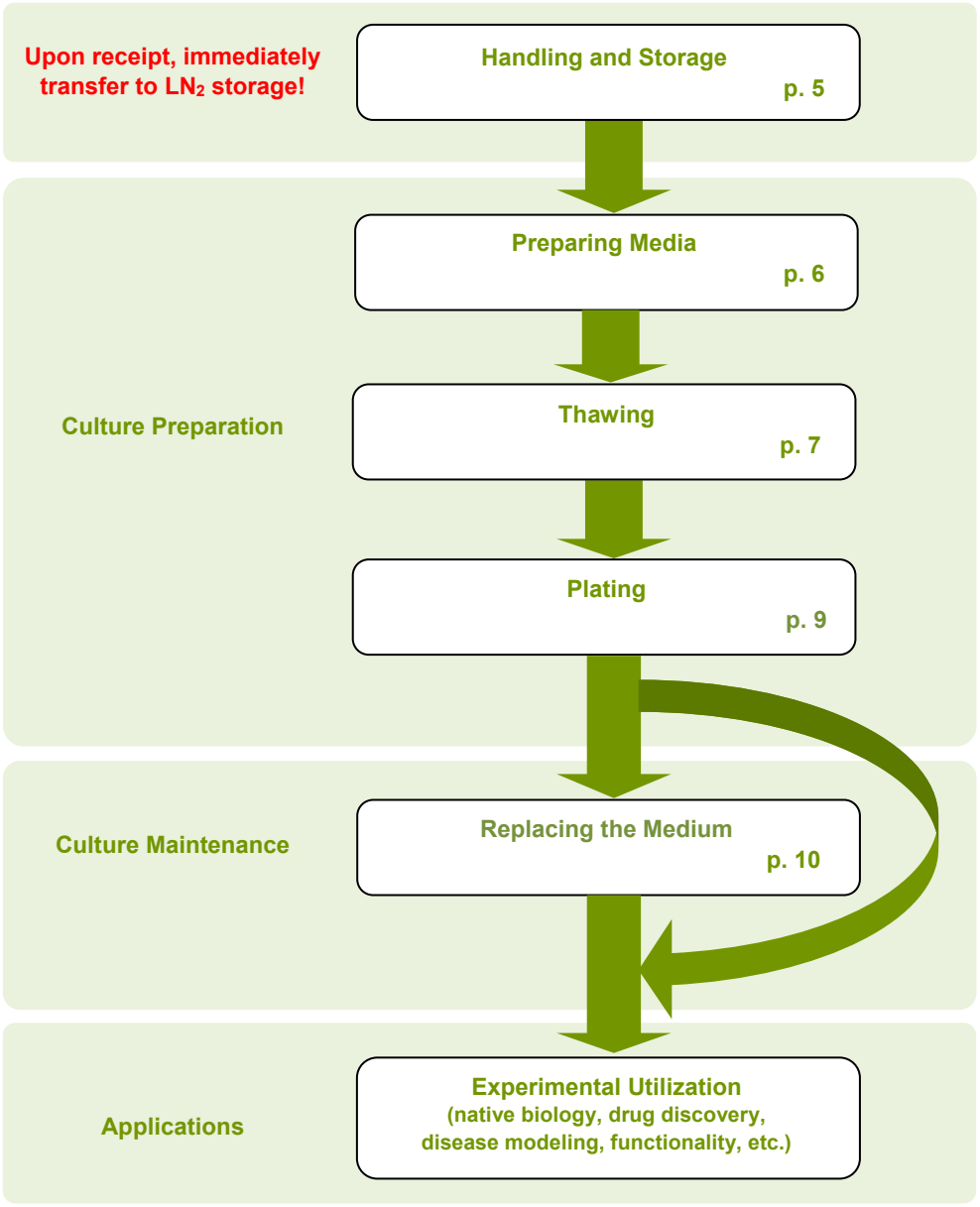
Technical Support and Training

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

Chapter 3. Preparing Media

Notes

iCell Macrophages thawing, plating, and maintenance require iCell Macrophages Thawing Medium (Thawing Medium) and iCell Macrophages Maintenance Medium (Maintenance Medium). Prepare using sterile technique and store as follows:

Thawing Medium*	
Component	**Final Concentration**
IMDM	90%
FBS	10%

* When preparing Thawing Medium, filter using a 0.2 µm PES filter unit. Store the medium at 4°C for up to 2 weeks.

Maintenance Medium*	
Component	**Final Concentration**
SFEM	99%
Glutamax	1%
ExCyte	0.3%
MCSF	20 ng/ml
IL1β	10 ng/ml

* When preparing Maintenance Medium, filter using a 0.2 µm PES filter unit. Store the medium at 4°C for up to 2 weeks.

Notes

Chapter 4. Thawing iCell Macrophages

iCell Macrophages have been demonstrated to plate and function on Nunclon Delta tissue culture vessels.

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

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

1. Equilibrate the Thawing Medium at room temperature before thawing iCell Macrophages.
2. Remove the iCell Macrophages 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 approximately 2 minutes (avoid submerging the cap) while gently 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 Macrophages 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 macrophage viability.*

   

   Avoid repeated pipetting of the thawed iCell Macrophages cell suspension.
6. Rinse the empty iCell Macrophages cryovial with 1 ml of room temperature Thawing Medium to recover any residual cells from the cryovial. Transfer the 1 ml Thawing Medium rinse from the cryovial drop-wise (~1 drop per second) to the 50 ml centrifuge tube containing the iCell Macrophages 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 Thawing Medium to the cell suspension is critical to minimize osmotic shock and ensure maximum viability.
7. Slowly add 8 ml of room temperature Thawing Medium to the 50 ml centrifuge tube. Add the first 1 ml drop-wise (~1 drop per second). Add the remaining 7 ml over the next minute. Gently swirl the centrifuge tube while adding the medium.

   

   It is critical to add the 8 ml of Thawing Medium slowly to ensure maximum viability of the cells.
8. Gently mix the contents of the 50 ml centrifuge tube by inverting once. Gentle mixing is critical to ensure maximum viability. Avoid vigorous shaking or vortexing of the cell suspension.

9. Centrifuge the cell suspension at 300 x g for 5 minutes at room temperature.
10. Aspirate the supernatant, leaving 1 ml in the centrifuge tube.
11. Gently resuspend the cell pellet in 5 ml of room temperature Maintenance Medium.

    Note: *CDI recommends using room temperature Maintenance Medium to resuspend the cells pellet to reduce the tendency of iCell Macrophages to attach to surfaces, which can be increased in 37°C medium.*

    Note: *Thaw up to 3 vials of iCell Macrophages at one time. However, each vial must be thawed according to the outlined procedure (i.e. use 9 ml of Thawing Medium for each vial: 1 ml for transferring residual cells and 8 ml for dilution). Once thawed and diluted to the desired density in Maintenance Medium, you can pool the cell suspensions for plating. When thawing more than one vial, work quickly while regularly swirling the cell container to avoid unintended attachment.*

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Chapter 5. Plating iCell Macrophages

The recommended plating density for iCell Macrophages is 50,000 viable cells/cm^2.

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. Dispense the cell suspension into the appropriate cell culture vessel(s).
4. Culture iCell Macrophages in a cell culture incubator at 37°C, 5% CO_2.

Expected Cell Density

50,000 viable cells/cm^2 is the recommended starting density of iCell Macrophages for most cell-based assays (Figure 2). However, the optimal density of iCell Macrophages per unit of surface area will 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 (5 x 10^4 cells/cm^2)
6-well Cell Culture Plate	9.6	2	480 x 10^3
12-well Cell Culture Plate	3.8	1	190 x 10^3
24-well Cell Culture Plate	1.9	0.5	95 x 10^3
96-well Cell Culture Plate	0.32	0.1	16 x 10^3

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

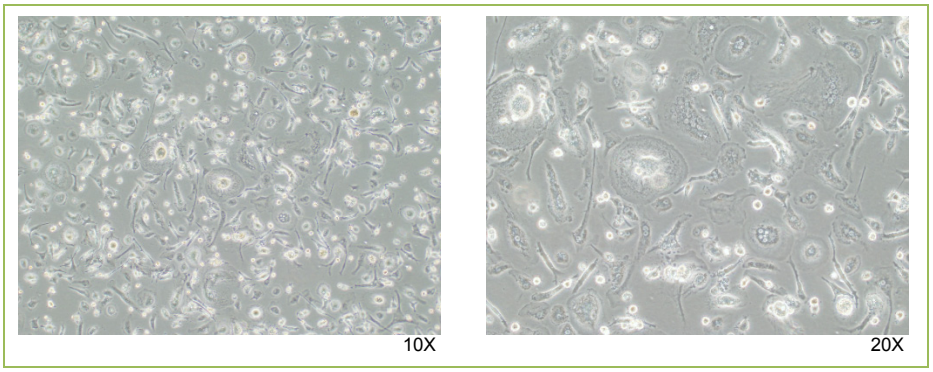

Figure 2: iCell Macrophages at 24 Hours Post-plating
These images show iCell Macrophages plated at 50,000 cells/cm^2 at 24 hours post-plating.

Chapter 6. Maintaining iCell Macrophages

Notes

iCell Macrophages are shipped cryopreserved at high purity. The cells preserve a high purity if maintained in prepared Maintenance Medium and cultured as recommended in a standard cell culture incubator (37°C, 5% CO_2).

1. Immediately before use, equilibrate the Maintenance Medium in a 37°C water bath. Do not equilibrate the Maintenance Medium in 37°C water bath multiple times. Aliquot the medium into small working volumes during cell maintenance.
2. 48 hours post-plating iCell Macrophages, aspirate the spent medium and replace with the appropriate volume of Maintenance Medium. Recommended volumes are as follows:
    - **6-well cell culture plate:** 2 ml/well
    - **12-well cell culture plate:** 1 ml/well
    - **24-well cell culture plate:** 0.5 ml/well
    - **96-well cell culture plate:** 0.1 ml/well
3. Replace the Maintenance Medium every 2 days.
4. Culture iCell Macrophages in a cell culture incubator at 37°C, 5% CO_2.

Notes

Appendices

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

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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.

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