FUJIFILM
Cellular Dynamics

iCell® Macrophages 2.0
User's Guide

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Conditions of Use

iCell Macrophages 2.0 are FOR RESEARCH USE ONLY and NOT FOR THERAPEUTIC USE. See www.fujifilmcdi.com/terms-and-conditions/ for USE RESTRICTIONS applicable to the cells and other terms and conditions related to the cells and their use.

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Origin

iCell Macrophages 2.0 are manufactured in the United States of America.

Copyright Notice

Revision History

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Version 1:0: November 2022

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Before You Begin

Notes

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire User's Guide before handling or using iCell® Macrophages 2.0.
- iCell Macrophages 2.0 are FOR RESEARCH USE ONLY and NOT FOR THERAPEUTIC USE. See www.fujifilmcdi.com/terms-and-conditions/ for USE RESTRICTIONS applicable to the cells and other terms and conditions related to the cells and their use.
- A Safety Data Sheet (SDS) for dimethyl sulfoxide (DMSO), in which iCell Macrophages 2.0 are frozen, is available online at www.fujifilmcdi.com/product-literature/ or on request from FUJIFILM Cellular Dynamics. Only technically qualified individuals experienced in handling DMSO and human biological materials should access, use, or handle iCell Macrophages 2.0.

Notes

Chapter 1. Introduction

iCell Macrophages 2.0 from FUJIFILM Cellular Dynamics, Inc. (FCDI), are highly purified, human macrophages derived from induced pluripotent stem (iPS) cells using FCDI's proprietary differentiation and purification protocols. iCell Macrophages 2.0 exhibit expected physiological characteristics and responses. iCell Macrophages 2.0 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.

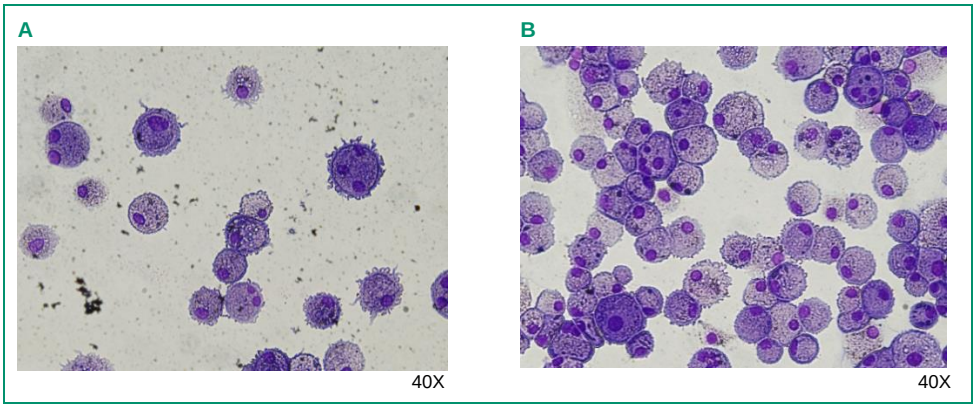

Figure 1: iCell Macrophages 2.0 Represent a Highly Pure Population of Human Macrophages
These images show iCell Macrophages 2.0, 01279 cytospins stained with Wright stain: (A) 10,000 cells per spot and (B) 50,000 cells per spot.

Components Supplied by FUJIFILM Cellular Dynamics

Item	Catalog Number
iCell Macrophages 2.0 Kit, 01279 •iCell Macrophages 2.0, 01279[1] •iCell Macrophages 2.0 User's Guide[1]	R1186 •C1193 (≥1.0 x 10^6 viable cells) •X1010
Certificate of Testing[2]	
Certificate of Origin If required for shipping purposes	

1 Safety Data Sheet and User's Guide available online: www.fujifilmcdi.com/product-literature/

2 Available online: www.fujifilmcdi.com/coa-lookup/

Required Equipment and Consumables

Item	Vendor(s)	Catalog Number(s)
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	
Phase Contrast Microscope	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
Conical Tubes, 15 ml, Falcon (Centrifuge Tubes)[2]	Multiple Vendors	
FBS	Hyclone	SH30396.03
Flat-bottom Plate, TC-treated[2,3,4]	Multiple Vendors	
Flat-bottom Plate, Ultra-low Attachment[3,4]	Corning	3471 (6 well) 3473 (24 well) 3474 (96 well)
Glutamax	Gibco	35050
Penicillin-Streptomycin (Optional)	Gibco	15140
IMDM	Thermo Fisher Scientific	12440
PES Filter Unit, 0.2 µm	Multiple Vendors	
Serological Pipettes, 5, 10, 25 ml[2]	Multiple Vendors	
SFEM	STEMCELL Technologies	09650
Trypan Blue[2]	Multiple Vendors	

1 Ensure the automated cell counter is appropriately calibrated before use.

2 Similar products are available from multiple vendors.

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

4 Cell culture plate coating assay dependent.

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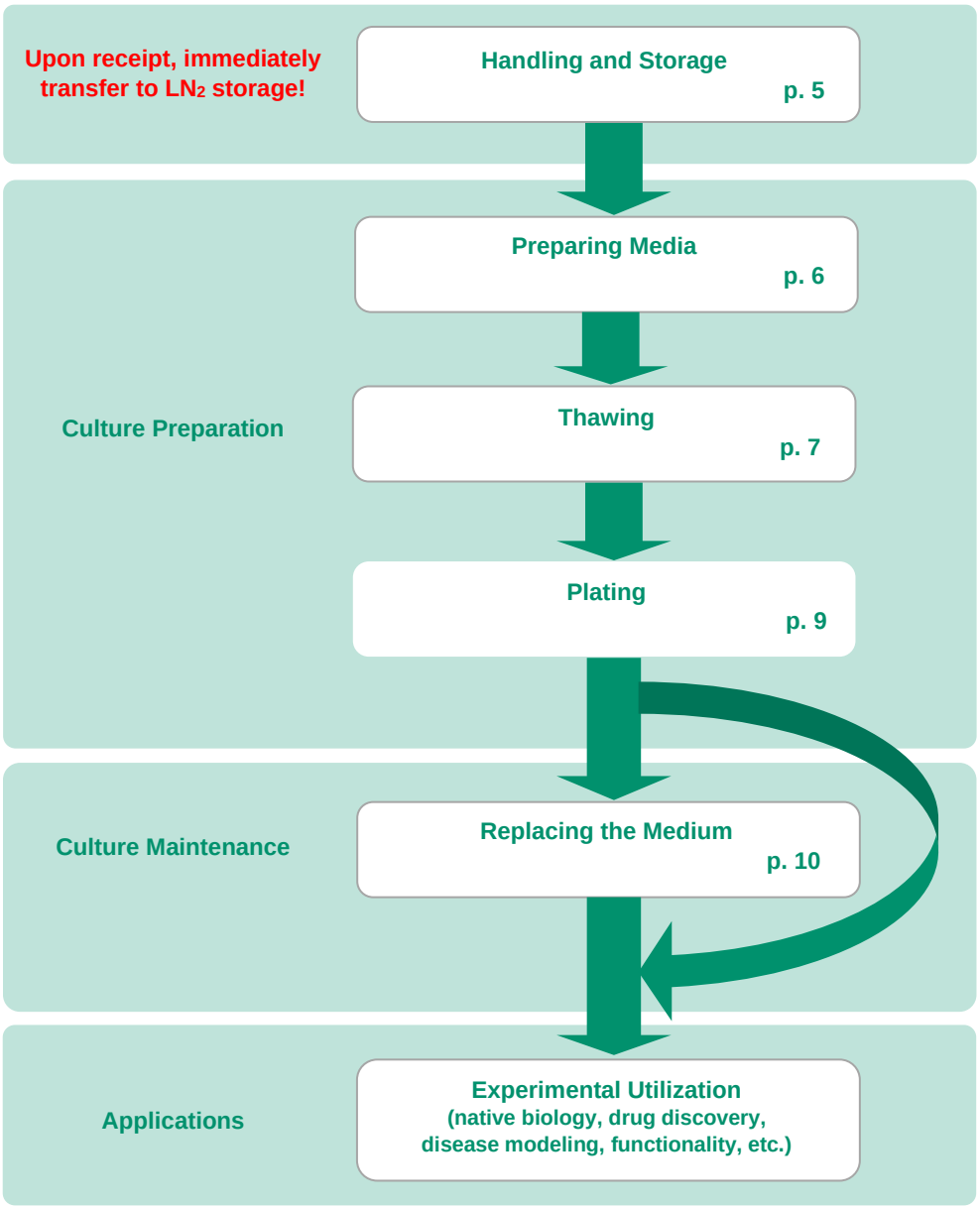
Technical Support, Knowledge Base, and Training

FCDI'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 x3 Monday - Friday, 8:30 am - 5:00 pm US Central Time
Email	fcdi-support@fujifilm.com
Knowledge Base	www.fujifilmcdi.com/knowledge-base/

Workflow Diagram



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Chapter 2. Handling and Storage

iCell Macrophages 2.0 are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the cryobox containing iCell Macrophages 2.0 to the vapor phase of a liquid nitrogen storage dewar. FCDI strongly recommends transferring the entire cryobox into the storage rack to avoid transferring individual vials.

It is critical to maintain cryopreserved iCell Macrophages 2.0 at a stable temperature. Minimize exposure of cryopreserved iCell Macrophages 2.0 to ambient temperature when transferring vials to liquid nitrogen storage.

Chapter 3. Preparing Media

Notes

Thawing, plating, and maintaining iCell Macrophages 2.0 require Macrophage Thawing Medium and Macrophage Maintenance Medium.

1. Prepare 10 ml of the Thawing Mediium according to Table 1:

Component	Volume (ml)	Final Concentration
IMDM	9	90%
FBS	1	10%

Table 1: Thawing Medium (10 ml)*

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

The Thawing Medium can be stored at 4°C for up to two weeks. The addition of Penicillin-streptomycin to the Thawing Medium is optional.

2. Prepare 100 ml of the Maintenance Mediium according to Table 2:

Component	Volume (ml)	Final Concentration
SFEM	98	98%
Glutamax	1	1%
Pen/Strep (Optional)	1	1%

Table 2: Maintenance Medium (100 ml)*

* When preparing Macrophage 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 2.0

iCell Macrophages 2.0 have been demonstrated to plate and function on tissue culture or ULA-treated cell culture vessels. The optimal vessel type will be assay dependent and must be determined empirically based on the intended use.

Maintain iCell Macrophages 2.0 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 Macrophages 2.0 viability and performance.

Note: *Thaw no more than 3 vials of iCell Macrophages 2.0 at one time.*

1. Equilibrate the Thawing Medium at room temperature before thawing iCell Macrophages 2.0.
2. Remove the iCell Macrophages 2.0 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 in a biological safety cabinet.
5. Gently transfer the iCell Macrophages 2.0 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 cell viability.*

   Avoid repeated pipetting of the thawed iCell Macrophages 2.0 cell suspension.
6. Rinse the empty iCell Macrophages 2.0 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 2.0 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 swirling 3-4 times. Gentle mixing is critical to ensure maximum viability. Avoid vigorous shaking or vortexing of the cell suspension.
9. Transfer the cell suspension to a 15 ml centrifuge tube.
10. Centrifuge the cell suspension at 300 x g for 5 minutes at room temperature.
11. Carefully aspirate the supernatant without disturbing the cell pellet.
12. Gently resuspend the cell pellet in an appropriate volume of room temperature Maintenance Medium by pipetting up and down 2 - 3 times.

    Note: *FCDI recommends using room temperature Maintenance Medium to resuspend the cells pellet to reduce the tendency of iCell Macrophages 2.0 to attach to surfaces, which can be increased in 37°C medium.*

    Note: *Thaw up to 3 vials of iCell Macrophages 2.0 at one time. However, each vial must be thawed according to the outlined procedure (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 2.0

The recommended plating density for iCell Macrophages 2.0 is 25,000-75,000 viable cells/cm^2 dependent on plate and application.

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 2.0 in a cell culture incubator at 37°C, 5% CO_2.

Expected Cell Density

25,000 – 75,000 viable cells/cm^2 is the recommended starting density of iCell Macrophages 2.0 for most cell-based assays (Figure 2). However, the optimal density of iCell Macrophages 2.0 per unit of surface area will be assay dependent and must be determined empirically based on the intended use. The following table (Table 3) 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	0.2	16 x 10^3

Table 3: Summary of Recommended Volumes and Measures
*All volumes and measures are **per well**.*

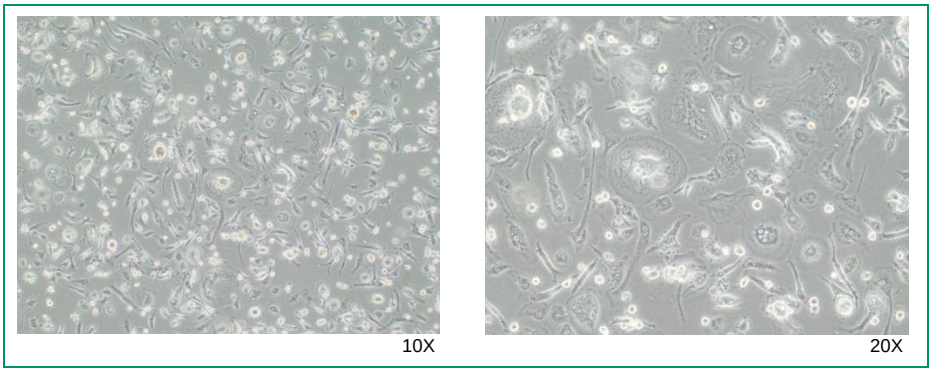

Figure 2: iCell Macrophages 2.0 at 24 Hours Post-plating
These images show iCell Macrophages 2.0, 01279 plated at 50,000 cells/cm^2 at 24 hours post-plating.

Chapter 6. Maintaining iCell Macrophages 2.0

Notes

iCell Macrophages 2.0 are shipped cryopreserved at high purity. The cells maintain a high purity if maintained inMaintenance 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.

   Note: *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 2.0, aspirate the spent medium and replace with the appropriate volume of Maintenance Medium. Recommended volumes are as follows:

Culture Vessel	Volume (ml)
6-well Cell Culture Plate	2
12-well Cell Culture Plate	1
24-well Cell Culture Plate	0.5
96-well Cell Culture Plate	0.2

Table 4: Summary of Recommended Volumes
*All volumes are **per well**.*

3. Replace the Maintenance Medium every 2 days.
4. Culture iCell Macrophages 2.0 in a cell culture incubator at 37°C, 5% CO_2.