



iCell[®] Microglia User's Guide

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

Conditions of Use

iCell Microglia are FOR RESEARCH USE ONLY. <https://fujifilmcdi.com/assets/tnc/standard.pdf> 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 Microglia 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 User's Guide before handling or using iCell® Microglia.
- iCell Microglia are FOR RESEARCH USE ONLY. See <https://fujifilmcdi.com/assets/tnc/standard.pdf> 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 Microglia are frozen, is available online at www.fujifilmcdi.com/lit/ 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 Microglia.

Chapter 1. Introduction

iCell Microglia from FUJIFILM Cellular Dynamics, Inc. (FCDI), are a highly pure population of human microglia derived from induced pluripotent stem (iPS) cells using licensed differentiation protocols based on the work of Abud, et al ([iPSC-Derived Human Microglia-like Cells to Study Neurological Diseases](#)). iCell Microglia exhibit expected physiological characteristics and responses. These cells provide a reliable source of human microglia suitable for use in targeted drug discovery, toxicity testing, and other life science research.

Components Supplied by FUJIFILM Cellular Dynamics

Item	Catalog Number
iCell Microglia Kit, 01279	R1131
<ul style="list-style-type: none"> •iCell Microglia, 01279¹ •iCell Microglia Quick Guide 	<ul style="list-style-type: none"> •C1110 ($\geq 1.0 \times 10^6$ viable cells) •X1022
Certificate of Analysis ²	
Certificate of Origin If required for shipping purposes	

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

2 Available online: www.fujifilmcdi.com/coa_lookup/

Required Equipment and Consumables

Item	Vendor(s)	Catalog Number(s)
Equipment		
37 °C Cell Culture Incubator	Multiple Vendors	
37°C Water Bath	Multiple Vendors	
Biological Safety Cabinet with UV Lamp	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
0.2 µm Sterile Filter Unit	Multiple Vendors	
1-Thioglycerol (MTG) 11.5 M	MilliporeSigma	M6145
Ascorbic Acid	FUJIFILM Wako Chemicals	013-19641
B-27 Supplement, 50X	ThermoFisher	175040
Bovine Serum Albumin	MilliporeSigma	A1470
Cell Culture Plates, Poly-D-lysine (PDL) Coated	Multiple Vendors	
Cell Culture-Treated Plates	Multiple Vendors	
Conical Tube, 15 ml Falcon (Centrifuge Tubes)	Corning	352196
Serological Pipettes, 1, 2, 5, 10, 25 ml	Multiple Vendors	
DMEM/F-12, HEPES, no phenol red	ThermoFisher	110390
Dulbecco's Phosphate Buffered Saline without Ca ²⁺ and Mg ²⁺ (DPBS)	ThermoFisher	14190
GlutaMAX Supplement	ThermoFisher	350500
Human Insulin Solution	MilliporeSigma	I9278
Insulin-Transferrin-Selenium, 100X	ThermoFisher	41400045
MEM Non-essential Amino Acids, 100X	ThermoFisher	111400
N-2 Supplement, 100X	ThermoFisher	175020
Penicillin-Streptomycin	ThermoFisher	15140
Recombinant Human CD200	ACRO Biosystems	OX2-H5228
Recombinant Human Fractalkine	PeproTech	300-31
Recombinant Human IL-34	PeproTech	200-34
Recombinant Human M-SCF	PeproTech	300-25
Recombinant Human TGF-β 1	R & D Systems	240-B

Technical Support, Knowledge Base, and Training

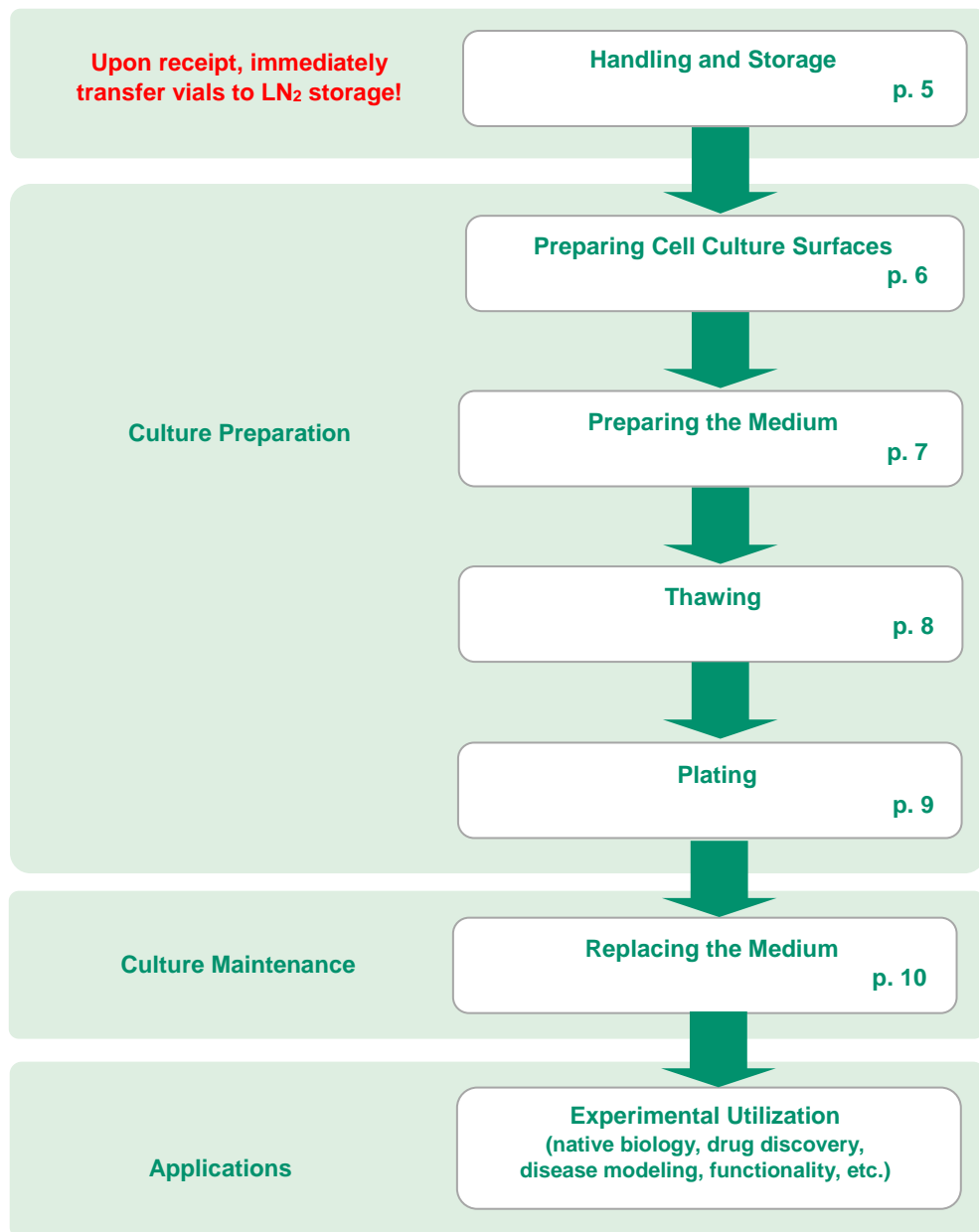
FCDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. Our web-based Knowledge Base provides solutions for iCell related questions about plating and media, cell culture, general assay methods, and more. In addition, in-lab training may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

Workflow Diagram



Chapter 2. Handling and Storage

iCell Microglia are provided as cryopreserved cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the iCell Microglia to the vapor phase of a liquid nitrogen storage dewar.



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

Chapter 3. Preparing Cell Culture Surfaces

iCell Microglia will plate and maintain function and viability on a variety of substrates. Cell culture surfaces may be assay-specific, but uncoated cell culture-treated and pre-coated Poly-D-Lysine cell culture plates are simple methods for culturing iCell Microglia.

iCell Microglia are highly mobile cells, able to shift shapes on different substrates. Other cell culture surfaces can influence adherence, morphology and function. Contact Technical Support for additional substrates amenable to the iCell Microglia.

Chapter 4. Preparing the Medium

FCDI recommends thawing, plating, and maintaining iCell Microglia in the following medium.

1. Prepare 10% BSA solution by diluting bovine serum albumin to 10% (w/v) in DPBS.
2. Spray all medium components with 70% ethanol and place in a biological safety cabinet.
3. Using sterile technique, combine the following components to prepare maintenance medium

Table 1: Components of Maintenance Medium for iCell Microglia

Component	Volume (ml)
DMEM/F-12, HEPES, no phenol red	93.3
B-27 Supplement, 50X	1
GlutaMAX Supplement	1
Insulin-Transferrin-Selenium, 100X	1
MEM Non-essential Amino Acids, 100X	1
Penicillin-Streptomycin	1
10% BSA Solution	0.5
N-2 Supplement, 100X	0.5
rhCD200, 100 µg/ml ¹	0.1
rhIL-34, 100 µg/ml ¹	0.1
rhFractalkine, 100 µg/ml ¹	0.1
Human Insulin Solution	0.05
rhTGF-β, 100 µg/ml ¹	0.05
Ascorbic Acid, 20 mg/ml ¹	0.25
rhM-CSF, 100 µg/ml ¹	0.025
1-Thioglycerol (MTG) 11.5 M	0.004

¹ Reconstitute according to manufacturer's recommendations

4. Filter maintenance medium using a 0.2 µm PES filter unit.
5. Store maintenance medium at 4°C for up to 2 weeks.
6. Single use aliquots may be stored at -20°C for up to 3 months.

Chapter 5. Thawing iCell Microglia

Maintain iCell Microglia in liquid nitrogen until immediately before thawing to ensure maximal performance of the cells. Completing the following steps in a time-efficient manner facilitates optimal iCell Microglia viability and performance.

1. Equilibrate the maintenance medium to room temperature before thawing iCell Microglia.
2. Transfer 8 ml of microglia maintenance medium to a sterile 15 ml centrifuge tube.
3. Remove the iCell Microglia cryovial from the liquid nitrogen storage tank.
Note: If necessary, transport cryovials on dry ice before thawing.
4. Thaw the cryovial in a 37°C water bath for 3 minutes (avoid submerging the cap). Use of a floating microcentrifuge tube rack is recommended.
5. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place in a biological safety cabinet.
6. Gently transfer iCell Microglia cryovial contents to the 15 ml centrifuge tube containing 8 ml maintenance medium using a 1 ml pipettor.
7. Rinse the empty iCell Microglia cryovial with 1 ml of room temperature maintenance medium to recover any residual cells from the cryovial.
8. Transfer the medium rinse from the cryovial to the 15 ml centrifuge tube containing the iCell Microglia suspension.
9. Gently mix the contents of the 15 ml centrifuge tube by inverting 2 - 3 times or slowly pipetting. Mixing is critical to ensure maximum viability.
10. Centrifuge the cell suspension at 1000 x g for 10 minutes.
11. Aspirate the supernatant, leaving 200 - 300 µl above the cell pellet to avoid disturbing the pellet.

Chapter 6. Plating iCell Microglia

The recommended plating density for iCell Microglia is ~50,000 viable cells/cm².

1. Obtain the number of total viable cells from the Certificate of Analysis.
2. Dilute the cell suspension using maintenance medium to obtain the desired cell plating density.
3. Invert the cell suspension 2 - 3 times or gently pipette to mix.
4. Immediately dispense the cell suspension to the cell culture vessel and allow to sit undisturbed for 15 - 20 minutes to allow the cells to adhere.
5. Culture iCell Microglia in a cell culture incubator at 37°C, 5% CO₂.

Expected Cell Density

iCell Microglia can be used at varying plating densities, ranging from ~10,000 - 100,000 viable cells/cm². However, the optimal density of iCell Microglia can be assay dependent and must be determined empirically based on the intended use. The following table provides the recommended cell number and plating volume for several common culture vessels when plating at a moderate density of 50,000 cells/cm².

Table 2: Summary of Recommended Volumes and Measures

Culture Vessel	Surface Area (cm ²)	Plating Volume (ml)	Cell Number (~50,000 cells/cm ²)	Cell Density (cells/ml)
6-well Cell Culture Plate	9.6	2	500,000	250,000
96-well Cell Culture Plate	0.32	0.1	15,000	150,000

All volumes and measures are **per well**.

Chapter 7. Maintaining iCell Microglia



Take care to avoid dislodging the iCell Microglia during medium exchange as the cells are loosely adherent and easily dislodge during culture handling.

Maintaining in 6-well Cell Culture Plates

1. Immediately before use, equilibrate the maintenance medium to room temperature.
2. Add 1 ml of maintenance medium to each well every 2 - 3 days.
3. Culture iCell Microglia in a cell culture incubator at 37°C, 5% CO₂.

Maintaining in 96-well Cell Culture Plates

1. Immediately before use, equilibrate the maintenance medium to room temperature.
2. Perform 50% medium exchanges every 2 - 3 days.
3. Culture iCell Microglia in a cell culture incubator at 37°C, 5% CO₂.