FUJIFILM Cellular Dynamics

Quick Guide

iCell® Microglia

Handling and Storage

Upon receipt, immediately transfer the cryovial to liquid nitrogen storage.

Cell Culture Surfaces

For most applications, use tissue culture vessels pre-coated with Poly-D-Lysine.

Other cell culture surfaces can influence adherence, morphology and function; refer to the iCell® Microglia User's Guide for more information and protocols.

Preparing the Maintenance Medium

1. Prepare maintenance medium (see **Table 1**).
2. Filter maintenance medium using a 0.2 µm PES filter unit.
3. Store maintenance medium at 4°C for up to 2 weeks.
4. Single-use aliquots can be stored at -20°C for up to 3 months.
5. Equilibrate maintenance medium to room temperature before use.

Thawing the Cells

1. Transfer 8 ml of maintenance medium to a 15 ml centrifuge tube.
2. Thaw iCell Microglia cryovial in a 37°C water bath for 3 minutes. Clean with 70% ethanol.
3. Transfer the cells to the 15 ml centrifuge tube containing 8 ml of maintenance medium.
4. Rinse the cryovial with 1 ml of maintenance medium and add it to the centrifuge tube.
5. Gently mix by inverting the centrifuge tube or slowly pipetting.
6. Centrifuge the cells at 1000 x g for 10 minutes.
7. Carefully remove the supernatant leaving approximately 200-300 µl above the pellet to avoid disturbing the cell pellet.

Plating the Cells

1. Dilute the cell suspension with maintenance medium to obtain the desired cell plating density using the total viable cells from the Certificate of Analysis. See table below for plating density examples.

Culture Vessel	Surface Area (cm^2)	Plating Volume (ml)	Cell Number
12-well cell culture plate	3.8	1.2	1.8×10^5
96-well cell culture plate	0.32	0.1	1.5×10^4

2. Dispense the cells into the cell culture vessel.
3. Culture the cells at 37°C, 5% CO_2.

Maintaining the Cells

1. Replace 50% of the medium every 2-3 days.
2. Culture the cells at 37°C, 5% CO_2.

Contacting Technical Support

Email: techsupport@stemcell.com

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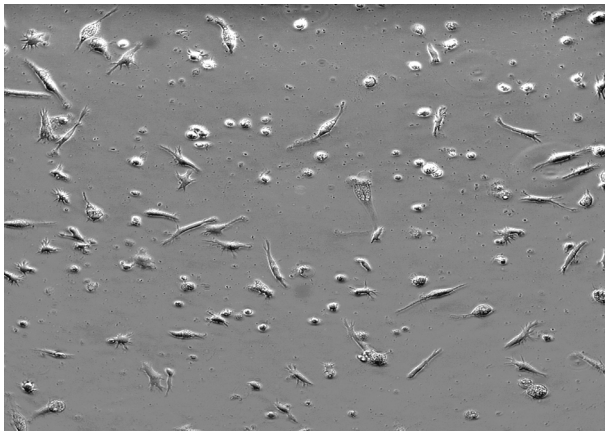

Figure 1: iCell Microglia cells, 20X

Table 1: Maintenance Medium Formulation for iCell Microglia

Component	Vendor Catalog #	Volume (ml)
DMEM/F-12, HEPES, no phenol red	ThermoFisher #110390	93.3
N-2 Supplement, 100X	ThermoFisher #175020	0.5
B-27 Supplement, 50X	ThermoFisher #175040	1
10% BSA in DPBS[1]	MilliporeSigma #A1470	0.5
1-Thioglycerol (MTG) 11.5M	MilliporeSigma #M6145	0.004
Ascorbic Acid, 20mg/ml[2]	FUJIFILM Wako Chemicals #013-19641	0.25
Penicillin-Streptomycin	ThermoFisher #15140	1
GlutaMAX Supplement	ThermoFisher #350500	1
MEM Non-essential Amino Acids, 100X	ThermoFisher #111400	1
Insulin-Transferrin-Selenium, 100X	ThermoFisher #41400045	1
Human Insulin Solution	MilliporeSigma #I9278	0.05
rhM-CSF, 100µg/ml[2]	PeproTech #300-25	0.025
rhTGF-ß 1, 100 µg/ml[2]	R&D Systems #240-B	0.05
rhIL-34, 100µg/ml[2]	PeproTech #200-34	0.1
rhFractalkine, 100 µg/ml[2]	PeproTech #300-31	0.1
rhCD200, 100 µg/ml[2]	Acro Biosystems #OX2-H5228	0.1

[1] DPBS no calcium, no magnesium, ThermoFisher #14190

[2] Reconstitute according to manufacturer's recommendations

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