FUJIFILM
Cellular Dynamics

Quick Guide

iCell® Microglia

Handling and Storage

Upon receipt, immediately transfer components to the proper storage temperature.

Component	Storage Temperature
iCell® Microglia, 01279 Cryovial	Vapor Phase of Liquid Nitrogen
iCell Glial Base Medium	4ºC
iCell Microglia Supplement A (100X)	-20ºC
iCell Microglia Supplement B (100X)	-20ºC
iCell Neural Supplement C (50X)	-20ºC

Preparing Cell Culture Surfaces

For most applications, use cell culture vessels pre-coated with Poly-D-Lysine. Contact Technical Support for assay-specific cell culture surface recommendations.

Preparing the Maintenance Medium

1. Thaw iCell Microglia Supplement A, iCell Microglia Supplement B, and iCell Neural Supplement C at room temperature.
2. Add the entire contents of iCell Microglia Supplement A (0.5 ml), iCell Microglia Supplement B (0.5 ml), and iCell Neural Supplement C (1 ml) to the iCell Glial Base Medium bottle (50 ml) to make the Complete Maintenance Medium.
3. Store Complete Maintenance Medium at 4°C for up to 1 week.
4. Equilibrate Complete Maintenance Medium to room temperature before use.

Thawing the Cells

1. Transfer 3 ml of Complete 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 3 ml of Complete Maintenance Medium.
4. Rinse the cryovial with 1 ml of Complete 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.

Centrifugation speed and time is critical for maximum cell recovery.

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 Complete 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.

   Note: *Optimal plating density may vary by application. See Table 3 for iCell Microglia Application Protocols.*

Culture Vessel	Surface Area	Plating Volume	Cell Number	Cell Density (viable cells/ml)
6-well Cell Culture Plate	9.6 cm^2	2 ml	500,000	250,000
96-well Cell Culture Plate	0.32 cm^2	100 µl	15,000	150,000

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

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

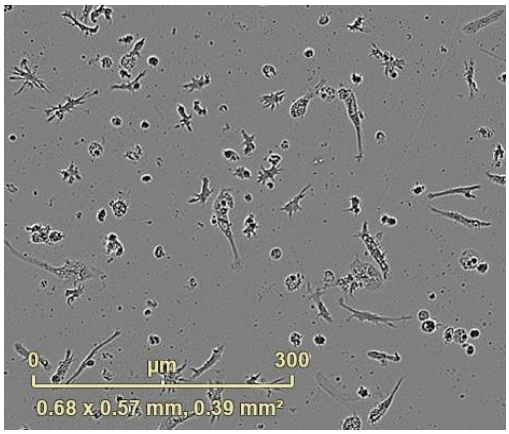

Figure 1: iCell Microglia, 01279

Table 1: iCell Microglia Complete Maintenance Medium[1]

Component	Catalog #	Volume
iCell Glial Base Medium	M1054	50 ml
iCell Microglia Supplement A (100X)	M1036	0.5 ml
iCell Microglia Supplement B (100X)	M1037	0.5 ml
iCell Neural Supplement C (50X)	M1055	1 ml

[1] *iCell Microglia may also be cultured according to Abud, et al (iPSC-Derived Human Microglia-like Cells to Study Neurological Diseases).*

Table 2: Cell Culture Vessel Recommendations

Component	Vendor	Catalog #
Cell Culture Multiwell Plate, 6-well Clear CELLCOAT, Poly-D-Lysine	Greiner Bio-One	657940
Cell Culture Microplate, µClear, 96-well Black CELLCOAT, Poly-D-Lysine	Greiner Bio-One	655946

Table 3: iCell Microglia Application Protocols

Application Protocols[1]
1. Immunofluorescent Labeling iCell Microglia
2. Measuring Microglia Phagocytosis: Kinetic Imaging on the IncuCyte
3. Tri-culture of iCell Microglia with iCell Neuronal Cell Types & iCell Astrocytes

[1] *Available at fujifilmcdi.com*

Contacting Technical Support

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Maintaining the Cells

Note: *It is recommended that iCell Microglia be allowed to recover from cryopreservation for a 3-day resting period prior to use in downstream assays.*

6-well Cell Culture Plates

1. Remove 1ml of spent Complete Maintenance Medium from each well and replace with 1ml of fresh medium every 2-3 days.
2. Culture the cells at 37°C, 5% CO_2.

96-well Cell Culture Plates

1. Replace 50% of the Complete Maintenance Medium from each well with an equal volume of fresh medium every 2-3 days.
2. Culture the cells at 37°C, 5% CO_2.

Avoid dislodging the iCell Microglia by aspirating and dispensing medium gently. The cells are loosely adherent and may detach during culture handling.

Revision History
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