

Handling and Storage

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

Component	Storage Temperature
iCell <sup>®</sup> Microglia, 01279 Cryovial	Vapor Phase of Liquid Nitrogen
iCell Glial Base Medium	4°C
iCell Microglia Supplement A (100X)	-20°C
iCell Microglia Supplement B (100X)	-20°C
iCell Neural Supplement C (25X)	-20°C

# **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.
- Add the entire contents of iCell Microglia Supplement A (0.5 ml), iCell Microglia Supplement B (0.5 ml), and two vials of 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.
- 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.
- Centrifuge the cells at <u>1000 x g</u> for <u>10 minutes</u>.



Centrifugation speed and time is critical for maximum cell recovery.

Carefully remove the supernatant leaving approximately 200-300 µl above the pellet to avoid disturbing the cell pellet.

# **Plating the Cells**

 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.

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

All volumes and measures are per well.

- Dispense the cells into the cell culture vessel allow to sit undisturbed for 15 20 minutes for the cells to adhere.
- **3**. Culture the cells at 37°C, 5% CO<sub>2</sub>.

**Quick Guide** 

# iCell<sup>®</sup> Microglia

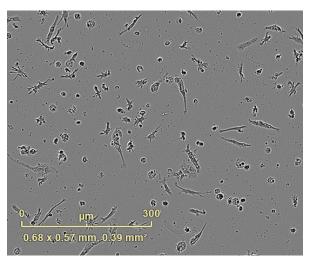


Figure 1: iCell Microglia, 01279

# Table 1: iCell Microglia Complete Maintenance Medium<sup>1</sup>

Component	Catalog #	Volume		
iCell Glial Base Medium	M1034	50 ml		
iCell Microglia Supplement A (100X)	M1036	0.5 ml		
iCell Microglia Supplement B (100X)	M1037	0.5 ml		
iCell Neural Supplement C (25X)	M1035	2 x 1 ml		
<sup>1</sup> iCell Microalia may also be cultured according to Abud, et al (iPSC-Derived				

Human Microglia-like Cells to Study Neurological Diseases).

# Table 2: Cell Surface 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

# iCell Microglia Application Protocols<sup>1</sup>

1. Immunofluorescent Labeling iCell Microglia

2. Measuring Microglia Phagocytosis: Kinetic Imaging on the IncuCyte

<sup>1</sup>Available at fujifilmcdi.com

# **Contacting Technical Support**

Email: fcdi-support@fujifilm.com

Phone: 1-877-320-6688



#### Maintaining the Cells



Avoid dislodging the iCell Microglia by dispensing medium gently as the cells are loosely adherent and easily detach during culture handling.

## 6-well Cell Culture Plates

- 1. Add 1 ml of Complete Maintenance Medium to each well every 2-3 days.
- 2. Culture the cells at 37°C, 5% CO<sub>2</sub>.

#### 96-well Cell Culture Plates

- 1. Replace 50% of the Complete Maintenance Medium every 2-3 days.
- 2. Culture the cells at 37°C, 5% CO<sub>2</sub>.

#### Conditions of Use

The cells are for RESEARCH USE ONLY. See <u>https://fujifilmcdi.com/assets/tnc/standard.pdf</u> for USE RESTRICTIONS applicable to the cells and other terms and conditions related to the cells and their use.

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#### **Revision History**

Document ID: X1022 Version 1.0 December 2018 Version 2.0 September 2019