User Guide

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Ricoh Biosciences, Inc.

Quick-Glia™ Microglia - Human iPSC-Derived Microglia

Catalog Numbers: MG-SeV-HC-CW50065

Introduction

Ricoh Biosciences' proprietary transcription factor-based technology allows rapid and reproducible differentiation of human iPSCs into microglia without sacrificing the purity of the cells. Our Quick-Glia™ Microglia - Human iPSC-derived microglia display typical microglia morphology and express markers such as allograft inflammatory factor 1 (AIF1; also called ionized calcium-binding adaptor molecule 1, IBA1), hexosaminidase subunit beta (HEXB), C-X3-C motif chemokine receptor 1 (CX3CR1), transmembrane protein 119 (TMEM119) and purinergic receptor P2Y12 (P2RY12). When handled and maintained according to the instructions in this user guide, the iPSC-derived microglia are viable long-term and are suitable for a variety of characterization and assays.

Scale: Each vial of Quick-Glia™ Microglia - Human iPSC-derived microglia is expected to have 1 million viable cryopreserved cells. The instructions outlined in this user guide are for seeding 1 million viable cells at approximately 1 x 10^5 cells/cm^2 into 1 well of a 6-well plate (1 x 10^6 cells/well), 5 wells of a 24-well plate (2 x 10^5 cells/well), or 33 wells of a 96-well plate (3 x 10^4 cells/well).

Related Products: Quick-Glia™ Microglia - SeV Kit, Catalog Number: MG-SeV (to be released soon)

Contents

Upon receipt, immediately store the items at the indicated temperatures. Be especially careful to keep the frozen cells on dry ice until placing them in liquid nitrogen and avoid any temperature fluctuation and slight thawing.

Contents	Amount	Storage	Thaw
Cryopreserved cells	>1 million viable cells, (1 vial, 500 µl)	Liquid nitrogen	37°C
Component MG1	55 µl	-20°C or -80°C	4°C
Component MG2	55 µl	-20°C or -80°C	Room Temperature
Component MG3	55 µl	-20°C or -80°C	Room Temperature

Condition of Use

This product is for research use only. It is not approved for use in humans or for therapeutic or diagnostic use.

Required Consumables

Item	Vendor	Catalog Number
(Optional) 6-well tissue-culture-treated polystyrene plate (e.g., Corning Costar Flat Bottom Cell Culture Plates)	Fisher Scientific	07-200-80
(Optional) 24-well tissue-culture-treated polystyrene plate (e.g., Corning Costar Flat Bottom Cell Culture Plates)	Fisher Scientific	07-200-740
(Optional) 96-well tissue-culture-treated polystyrene plate (e.g., PhenoPlate 96-well, black, optically clear flat-bottom)	Perkin Elmer	6055302
ScienCell Microglia Medium Kit: • Basal Medium • Microglia Growth Supplement (MGS) • Fetal bovine serum (FBS) • Penicillin-Streptomycin (P/S)	ScienCell Research Laboratories	1901
Fibronectin, human plasma, 0.1%	Sigma Aldrich	F0895
Phosphate-buffered saline (without Ca^{++} Mg^{++})*	ThermoFisher	20012050
Distilled Water	Fisher Scientific	15-230-001

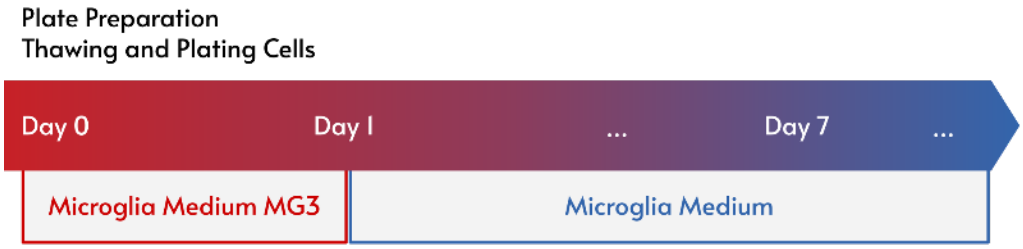
*PBS should be used at room temperature unless otherwise specified.

Technical Support

For technical support please refer to the FAQ on our website.
You may also contact us at cs@biosciences.ricoh.com or call +1 (443) 869-5420 (M-F 9am-5pm EST).

Workflow

Note: This protocol assumes that Day 0 is a Tuesday.



*From Day 7, users may maintain differentiated microglia in the maintenance medium best suited for their needs, though we recommend Microglia Medium.

Experiment Planning

Define the cell culture plate or dish format and calculate the number of wells to be used for each format in advance. For example, you may use only a certain number of wells of a 96-well plate. The following section describes culture volumes per well as users' needs may vary. When a 96-well plate is used, we recommend filling the edge wells of the plate with an aqueous medium (e.g., distilled water) instead of cells and culture medium. This will maintain humidity on the entire plate. If performing image-based analysis with a 96-well plate, we have found plating approximately 3 x 10^4 cells/well to yield the best results. Please refer to the table below for plate formats and corresponding surface area of each well used for calculating reagents in the following sections.

Plate format	6-well plate	24-well plate	96-well plate
Approximate cell growth surface area per well	9.5 cm^2	1.9 cm^2	0.32 cm^2
Recommended plating viable cells per well	1 x 10^6 cells	2 x 10^5 cells	3.0 x 10^4 cells

Media Preparation

10 µg/ml fibronectin

1. Prepare 10 µg/ml fibronectin using the reagents listed in the table below.
    - Briefly spin down 0.1% fibronectin before use.
    - Store undiluted fibronectin at 4°C.

10 µg/ml fibronectin	Volume
0.1% fibronectin, human plasma	60 µl
PBS	6 ml

ScienCell Medium

1. Prepare ScienCell Medium using the reagents listed in the table below.
    - Warm basal medium, fetal bovine serum (FBS), microglia growth supplement (MGS), and penicillin-streptomycin (P/S) from the ScienCell kit according to manufacturer's instructions.
        - **IMPORTANT!** MGS must be thawed at 37°C to become a thoroughly dissolved homogeneous solution.
    - Aliquot and store unused FBS and MGS at -20°C and the Basal Medium and P/S at 4°C.

ScienCell Medium	Volume
Basal Medium	30 ml
FBS	1.5 ml
MGS	300 µl
P/S	300 µl

Microglia Medium

1. Prepare Microglia Medium using the reagents listed in the table below.
    - Warm ScienCell Medium for 20-30 minutes at room temperature.
    - Thaw Component MG1 for 20-30 minutes at 4°C.
    - Thaw Component MG2 for 20-30 minutes at room temperature.
    - Briefly spin down these Components before use.
    - Keep Microglia Medium, and any subsequent media made with it, protected from light.
    - Store Microglia Medium for up to 2 weeks at 4°C.
    - Keep leftover Sciencell Medium at 4°C for use on Day 0 when cells are thawed.
    - Leftover Components can be discarded or saved at 4°C for up to two weeks.

Microglia Medium	Volume
ScienCell Medium	25 ml
Component MG1	50 µl
Component MG2	50 µl

Microglia Medium MG3

1. Prepare Microglia Medium MG3 using the reagents listed in the table below.
    - Warm Microglia Medium for 20-30 minutes at room temperature.
    - Thaw Component MG3 for 20-30 minutes at room temperature.
    - Briefly spin down the Component before use.
    - Leftover Component MG3 can be discarded or saved at 4°C for up to two weeks.

Microglia Medium MG3	Volume
Microglia Medium	5 ml
Component MG3	5 µl

Day 0

5-6 hours

Plate Preparation

IMPORTANT! Cells can be plated in 6-well, 24-well, or 96-well plates depending on the desired format. Refer to the table at the beginning of the next page for the recommended volumes per well.

1. Calculate the required volume of 10 µg/ml fibronectin by multiplying the number of wells by the required volume per well as written in the table and add 10% extra. Aliquot the calculated volume of fibronectin into a new tube and keep on ice.
2. Add 10 µg/ml fibronectin to each well in the volume specified in the table below.
3. Incubate the plate at room temperature for at least 3 hours (or at 4°C overnight on the day before plating).
4. While the plate is incubating, warm ScienCell medium and Microglia Medium MG3 at room temperature for 20-30 minutes.
5. After the fibronectin incubation, aspirate most, but not all, of the supernatant.
6. Add Microglia Medium MG3 to each well in the volume specified in the table.
7. Incubate the plate at 37°C, 5% CO_2 until cells are ready for plating.

Reagents	Corresponding steps	Recommended volume per well		
		6-well plate	24-well plate	96-well plate
10 µg/ml fibronectin	1,2	4.75 ml	950 µl	160 µl
Microglia Medium MG3	6	1.5 ml	300 µl	50 µl

Thawing Cells

1. Take out the vial of frozen cells from the liquid nitrogen storage tank.
2. Incubate the cryovial in a water bath set at 37°C (do not submerge the cap) until the most of the content is thawed but a small ice crystal remains (~2 minutes).
3. Wipe the vial with a dry paper towel. Spray 70% ethanol to the vial and bring it inside a biosafety cabinet.
4. Transfer 4.5 ml room temperature ScienCell Medium to a new 15 ml conical tube.
5. Set a P1000 pipette to 1 ml but take approximately 500 µl ScienCell Medium from the 15 ml conical and add it to the cryo-vial dropwise at 1 drop per 1-2 seconds.
    - **IMPORTANT!** Use the same pipette tip for Steps 6-10.
6. Gently pipet the cell suspension up and down once.
7. Transfer all of the cell suspension dropwise to the 15 ml conical tube prepared in Step 5.
8. Take 1 ml of the cell suspension from the conical tube and add it to the original cryovial and pipet up and down 2-3 times and then transfer the whole contents back to the same 15 ml conical tube.
9. Mix the contents in the conical tube by gently pipetting cell suspension up and down 3 times.
10. Centrifuge the cell suspension at 200 x g for 4 minutes.
11. Use an aspirator to remove most of the supernatant from the conical tube, leaving a small volume of the supernatant (<50 µl) to cover the pellet.
12. Tap the side of the conical tube up to 10 times to break up the cell pellet.
13. Add 1 ml room temperature Microglia Medium MG3 to the conical tube using a P1000 pipettor and pipet up and down no more than 2-3 times.

Plating Cells

1. Count the cells to determine the volume of cell suspension needed for the chosen number of wells and include 10% extra for cell number and volume (e.g., for a 24-well plate scenario, a total of 1.1 x 10^6 cells to plate 2 x 10^5 cells in each of the 5 wells). If the volume of the cell suspension needs to be adjusted, centrifuge the required volume of cell suspension at 200 x g for 4 minutes, remove the supernatant, and resuspend the pellet with ScienCell Medium to reach the multiplied volume of cell suspension with the number of wells.
2. Add cell suspension to the center of each well. Since each well already has Microglia Medium MG3, the total volume of the medium in each well is indicated in the table below.

	Recommended amounts		
	6-well plate	24-well plate	96-well plate
Viable cells per well	1 x 10^6 cells	2 x 10^5 cells	3 x 10^4 cells
Required total volume of cell suspension/well • (Volume of cell suspension/well) + 10% extra	1.65 ml	330 µl	55 µl
Volume of cell suspension distributed/well	1.5 ml	300 µl	50 µl
Total volume/well • Microglia Medium MG3 + cell suspension	3 ml	600 µl	100 µl

3. Move the plate in 5 cycles of quick back-and-forth and side-to-side motions to evenly distribute cells in the cultures.
4. Incubate at 37°C, 5% CO_2 overnight.

Day 1

< 1 hour

Maintenance

1. Warm Microglia Medium at room temperature for 20-30 minutes.
2. Pipet out the old medium from each well, leaving a small volume behind to avoid the cells drying out.
3. Add Microglia Medium to each well according to the table below.

	Required volume per well		
Reagents	6-well plate	24-well plate	96-well plate
Microglia Medium	6 ml	1.2 ml	200 µl

4. Incubate the culture at 37°C, 5% CO_2 for 2 days.

Days 3-6

< 1 hour

Maintenance

1. Warm Microglia Medium at room temperature for 20-30 minutes.
2. Pipet out half of the old medium from each well.
3. Add Microglia Medium to each well according to the table below.

	Required volume per well		
Reagents	6-well plate	24-well plate	96-well plate
Microglia Medium	3 ml	600 µl	100 µl

4. Incubate the plate at 37°C, 5% CO_2.
5. Repeat steps 1-4 every 2-3 days.

Day 7

< 1 hour

Assay or Continuous Maturation

- Cells will be ready for assays such as phagocytosis assay using pHrodo™ (ThermoFisher Scientific Cat#P35364), qRT-PCR and immunofluorescence microscopy.
- AIF1, HEXB, CXCR1, TMEM119, and P2RY12 can be detected in Day 7 culture by either immunological staining (for AIF1 and TMEM119) or qRT-PCR (all markers listed).
- From Day 7, users may maintain differentiated microglia in the maintenance medium best suited for their needs, though we recommend Microglia Medium.

Appendix

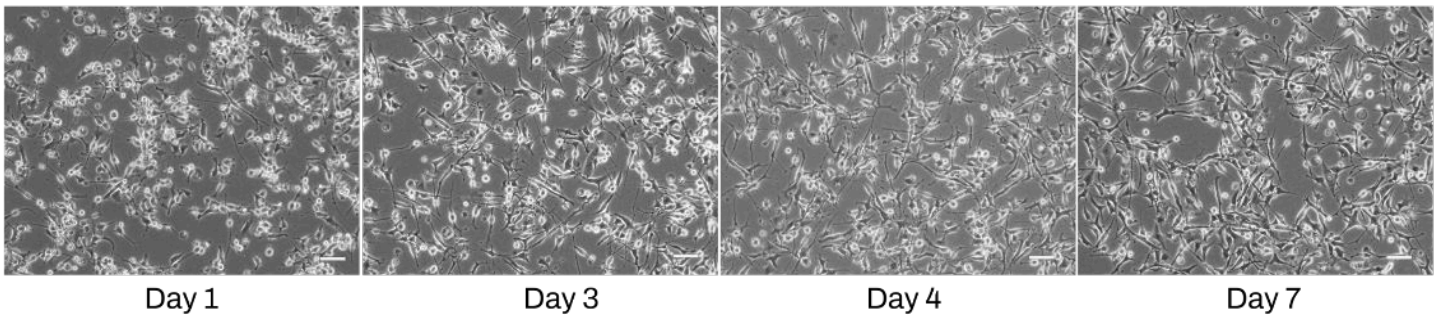


Figure 1. Representative images of Quick-Glia™ Microglia - Human iPSC-derived microglia on days 1 and day 7 post-thaw (scale bars = 100 µm).

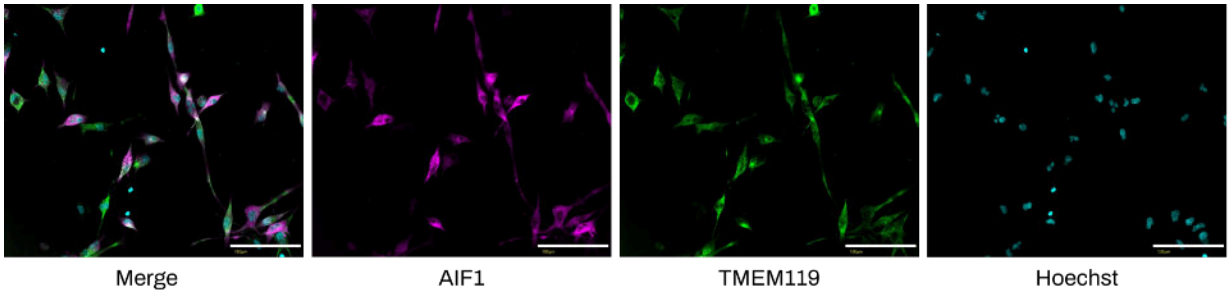


Figure 2. Immunofluorescent staining of Quick-Glia™ Microglia - Human iPSC-derived microglia culture has cells with typical amoeboid morphology and shows the expression of AIF1 (magenta) and TMEM119 (green) on day 7 post-thaw (scale bars = 100 µm). Staining conditions: the antibody against AIF1 (anti-Iba1 monoclonal antibody, Fujifilm Wako Pure Chemical Corporation, Catalog Number: 018-28523, 1:1200 dilution) was used in combination with a secondary antibody (ThermoFisher, Catalog Number: A32733, Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647, 1:500 dilution). Anti-TMEM119 antibody (Cell Signaling Technology, Catalog Number: 41134, 1:500 dilution) was used in combination with a secondary antibody (ThermoFisher, Catalog Number: A32723 Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, AlexaFluor Plus 488 1:500 dilution). Nuclei were counterstained with Hoechst 33342.