User Guide

Ricoh Biosciences, Inc.

Quick-Glia™ Astrocyte - Human iPSC-Derived Astrocytes

Catalog Numbers: AS-SeV-HC-CW50065

Introduction

Ricoh Biosciences' proprietary transcription factor-based technology allows rapid and reproducible differentiation of human iPSCs into astrocytes without sacrificing the purity of the cells. Our Quick-Glia™ Astrocyte - Human iPSC- derived astrocytes display typical astrocyte morphology and express markers such as S100 Calcium Binding Protein β (S100β), Chondroitin Sulfate Proteoglycan 8 (CD44), Aldehyde Dehydrogenase 1 Family Member L1 (ALDH1L1), and mature astrocyte marker Glial Fibrillary Acidic Protein (GFAP). When handled and maintained according to the instructions in this user guide, the iPSC-derived astrocytes are viable long-term and are suitable for a variety of characterization and assays.

Scale: Each vial of Quick-Glia™ Astrocyte - Human iPSC-Derived Astrocytes 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 2.6 x 10^4 cells/cm^2 into 2 wells of a 6-well plate (5 x 10^5 cells/well), 10 wells of a 24-well plate (1 x 10^5 cells/well), or 62 wells of a 96-well plate (1.6 x 10^4 cells/well).

Related Products: Quick-Glia™ Astrocyte - SeV Kit, Catalog Number: AS-SeV

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 P	50 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.

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).

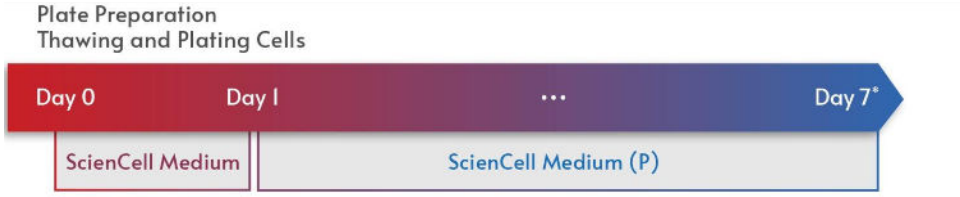
Required Consumables

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., Thermo Scientific™ 96 Well Black/Clear Bottom Plate)	Fisher Scientific	12-566-70
ScienCell Astrocyte Medium Kit: • Basal Medium • Astrocyte Growth Supplement • FBS • P/S	ScienCell Research Laboratories	1801
Geltrex hESC-Qualified, Ready-To-Use, Reduced Growth Factor Basement Membrane Matrix	ThermoFisher	A1569601
(Optional) Phosphate-buffered saline (without Ca^{++} Mg^{++})*	ThermoFisher	20012050
(Optional) TrypLE Select Enzyme (1X)	ThermoFisher	12563011
(Optional) 0.02% EDTA in DPBS	Sigma-Aldrich	E8008-100ML
(Optional) STEM-CELLBANKER**	AMSBIO	11890

*PBS should be used at room temperature unless otherwise specified.
**This is only required if you intend to cryopreserve the cells after differentiation.

Workflow

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



*From Day 7, users may maintain differentiated astrocytes in the maintenance medium best suited for their needs, though we recommend ScienCell Medium (without FBS and with the addition of Component P).

Experiment Planning

Define the cell culture plate or dish format in advance 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 condition volumes per well as user needs may vary. When a 96-well plate is used, we recommend filling the edge wells of the plate with an aqueous medium instead of cells and culture medium. This will maintain humidity on the entire plate. If performing an image-based analysis with a 96-well plate, we have found plating approximately 1-2 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	5 x 10^5 cells	1 x 10^5 cells	1.6 x 10^4 cells

Preparation

ScienCell Medium

1. Prepare ScienCell Medium using the reagents listed in the table below.
    - Warm Basal Medium, Astrocyte Growth Supplement (AGS), and Pen/Strep (P/S) from the ScienCell kit according to manufacturer's instructions.
    - Aliquot and store unused AGS at -20°C and the Basal Medium and P/S at 4°C.

Reagents	Volume
Basal Medium	29.5 mL
AGS	300 µL
P/S	300 µL

Note: Although the ScienCell Astrocyte Medium Kit includes FBS, do not add it to the media and instead culture Quick-Glia™ Astrocytes without FBS.

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 bottom of this page for the recommended volumes per well.

1. Calculate the required volume of Geltrex 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 Geltrex into a new tube and keep on ice.
2. Add Geltrex to each well in the volume specified in the table below.
3. Incubate the plate at 37°C, 5% CO_2 for at least 1 hour (or at 4°C overnight one day before plating).
4. While the plate is incubating, warm ScienCell Medium at room temperature for 20-30 minutes.
5. After the Geltrex incubation, aspirate most, but not all, of the supernatant.
6. Add ScienCell Medium 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
Geltrex	1,2	1.5 mL	300 µL	50 µL
ScienCell Medium	6	1 mL	400 µL	35 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. Gently transfer all of the cell suspension 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 ScienCell Medium 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 1 x 10^5 cells in each of the 10 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 ScienCell Medium, 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	5 x 10^5 cells	1 x 10^5 cells	1.6 x 10^4 cells
Required total volume of cell suspension/well • (Volume of cell suspension/well) + 10% extra	550 µl	110 µl	16.5 µl
Volume of cell suspension distributed/well	500 µl	100 µl	15 µl
Total volume/well • ScienCell Medium + cell suspension	1 ml	300 µl	50 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. Prepare ScienCell Medium (P) using the reagents listed in the table below.
    - Thaw Component P for 20-30 minutes at the temperature indicated in the "Contents" table on page 1.
    - Warm ScienCell Medium at room temperature for 20-30 minutes.
    - Tap the tube of Component P 3 times and then briefly spin down before use.

Reagents	Volume
ScienCell Medium	40 mL
Component P	20 µL

2. Pipet out the old medium from each well, leaving a small volume behind to avoid the cells drying out.
3. Add ScienCell Medium (P) to each well according to the table below.

	Required volume per well		
Reagents	6-well plate	24-well plate	96-well plate
ScienCell Medium (P)	2.5 mL	500 µL	100 µL

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

Days 3-6

< 1 hour

Maintenance

1. Warm ScienCell Medium (P) 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 ScienCell Medium (P) to each well according to the table below.

	Required volume per well		
Reagents	6-well plate	24-well plate	96-well plate
ScienCell Medium (P)	2.5 mL	500 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

- CD44, S100β, GFAP, and ALDH1L1-positive cells can be detected on Day 7. For more mature astrocytes with increased expression of GFAP and ALDH1L1, we recommend culturing cells until Day 14.
- From Day 7, users may maintain differentiated astrocytes in the maintenance medium best suited for their needs, though we recommend ScienCell Astrocyte Medium (without FBS and without the addition of Component P).