User Guide

Quick-Glia™ Astrocyte - SeV Kit (Large)

Catalog Number: AS-SeV-L

Introduction

The Quick-Glia™ Astrocyte - SeV Kit (Large) facilitates rapid and efficient differentiation of human iPS or ES cells into astrocyte cells in just 28 days. Our proprietary transcription factor-based stem cell differentiation method uses the Sendai virus to produce astrocytes without a genetic footprint. Quick-Glia™ Astrocyte differentiated cell cultures display typical astrocyte morphology and 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, astrocytes are viable long-term and are suitable for a variety of characterization and assays.

Scale: The Quick-Glia™ Astrocyte - SeV Kit (Large) contains a set of reagents for use with a total of 6 wells of a 6-well plate.

Related Products: Quick-Glia™ Astrocyte - SeV Kit (Small), Catalog Number: AS-SeV-S
Quick-Glia™ Astrocyte - Human iPSC-derived Astrocytes, Catalog Number: AS-SeV-CW

Contents

Upon receipt, store the reagents at the temperatures indicated in the table below. All reagents are shipped on dry ice.

Contents	Volume	Storage	Thaw
QGA-SeV (undiluted)*	100 µl	-80°C	On ice
Component N1	2 x 830 µl	-20°C or -80°C	On ice or 4°C
Component GA1	65 µl	-20°C or -80°C	On ice or 4°C
Component GA2	65 µl	-20°C or -80°C	Room Temperature

***IMPORTANT!** This kit contains Sendai virus (SeV) particles that are active at 33°C and become inactive at 37°C. SeV is non-pathogenic in humans, and humans are not natural hosts of SeV; however, Biosafety Level 2 (BSL-2) containment is required for its use. Please use a biological safety cabinet, laminar flow hood, and proper personal protective equipment in order to prevent mucosal exposure. More information on BSL-2 guidelines can be found at www.cdc.gov/labs/BMBL.html.

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@elixirgensci.com or call +1 (443) 869-5420 (M-F 9am-5pm EST).

Required Consumables

Item	Vendor	Catalog Number
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
DMEM/F12	ThermoFisher	21331020
Neurobasal Medium	ThermoFisher	21103049
GlutaMAX	ThermoFisher	35050061
Penicillin-Streptomycin	ThermoFisher	15140122
iMatrix-511 silk	Elixirgen Scientific	NI511S
ScienCell Astrocyte Medium Kit: • Basal Medium • Astrocyte Growth Supplement • FBS • P/S	ScienCell Research Laboratories	1801
TrypLE Select Enzyme (1X)	ThermoFisher	12563011
0.02% EDTA in DPBS	Sigma-Aldrich	E8008-100ML
Phosphate-buffered saline (without Ca^{++} Mg^{++})*	ThermoFisher	20012050
ROCK inhibitor Y27632	Selleckchem	S1049
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D2650
Geltrex hESC-Qualified, Ready-To-Use, Reduced Growth Factor Basement Membrane Matrix	ThermoFisher	A1569601
(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.

Source hPSC Culture Conditions

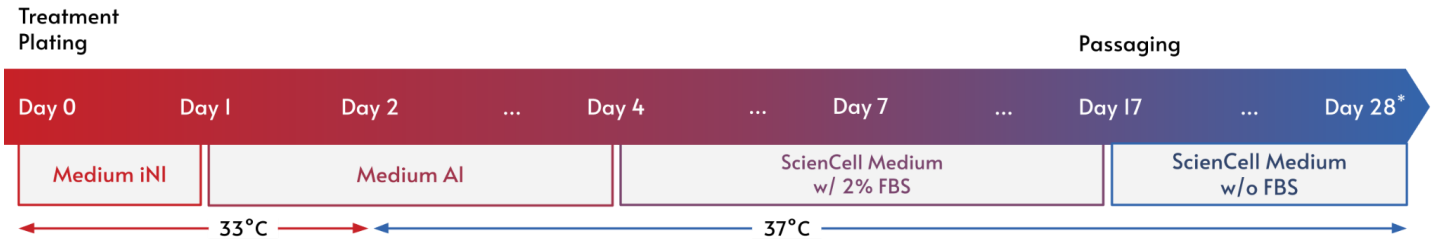
The Quick-Glia™ Astrocyte - SeV Kit (Large) gives the best differentiation results when source human pluripotent stem cells (hPSCs) have been maintained in StemFit® Basic04, StemFit® AK02N, StemFlex™ Medium, or other similar culture media which enable the maintenance of cultures by single-cell passaging. This protocol also assumes that the source hPSCs are cultured in a 35-mm culture dish or one well of a 6-well plate. If iMatrix-511 silk is routinely used as a coating substrate, prepare culture dishes or wells precoated with 0.25 µg/cm^2 iMatrix-511 silk diluted in 2 ml chilled PBS per well or dish for this kit.

- The protocols and reagents for StemFit® Basic04 and iMatrix-511 silk culture conditions are available at Elixirgen Scientific (Catalog Numbers: ASB04-C, NI511S).
- Differentiation should not be performed until the cells are at least 14 days post-thaw.
- We recommend preparing a minimum of 2 x 10^6 viable hPSC for use with this kit. This is usually obtained by using 2 wells of a 6-well plate at 50-70% confluency.
- For optimal differentiation, hPSC confluency should be around 50% to 70%. Do not use wells more than 90% confluent.

Workflow

IMPORTANT! This workflow requires a humidified 33°C, 5% CO_2 incubator. Before starting this protocol, please make sure the temperature is stable at 33°C.

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



* From Day 28, users may maintain differentiated cells in the maintenance medium best suited for their needs. We recommend continuing with the ScienCell Medium without FBS for an additional 2 weeks for more mature astrocytes.

Media Preparation

10 mM ROCK inhibitor Y27632 (iROCK)

1. Dissolve 10 mg ROCK inhibitor Y27632 in 3.12 ml DMSO.
2. Make aliquots of a convenient volume (e.g., 100 µl).
3. This solution, hereafter referred to as iROCK, can be stored at -20°C.

0.5X TrypLE Select with EDTA (Solution D1)

1. Mix 1.5 ml TrypLE Select Enzyme (1X) with 1.5 ml 0.02% EDTA in DPBS.
2. This mixture, hereafter referred to as Solution D1, can be stored at 4°C for 2 weeks.

Medium N1

1. Prepare Medium N1 using the reagents listed in the table below.
    - Thaw Component N1 for 20-30 minutes at the temperatures indicated in the “Contents” table on page 1.
    - Warm all other reagents at room temperature for 20-30 minutes.
    - Tap each Component tube 3 times and then briefly spin them down before use.
    - Keep Medium N1, and any subsequent media made with it, protected from light.
    - Store Medium N1 for up to 2 weeks at 4°C.
    - Leftover reagents can be discarded or saved at 4°C for up to 2 weeks.

Reagents	Volume
DMEM/F12	21.5 ml
Neurobasal	21.5 ml
GlutaMAX	225 µl
Penicillin-Streptomycin (10000 units/ml; 100x)	450 µl
Component N1	1.35 ml

Day -3

~4 hours

Note: This protocol assumes that Day 0 is a Monday so Day -3 is Friday.

Plate Preparation

1. Prepare diluted iMatrix-511 silk by mixing together the following components in a 15 ml conical tube.
    - Keep iMatrix-511 silk on ice.
    - Make sure chilled PBS is used for this mixture.

Reagents	Volume
iMatrix-511 silk	44.6 µl
Chilled PBS	13.5 ml

2. Add 2 ml diluted iMatrix-511 silk to each new well of a new 6-well plate.
3. Incubate the plate at 4°C.
   Note: For best results we recommend precoating the plate 1 day or up to a week before use and keeping at 4°C. Alternatively plates can be precoated on Day 0 and placed at 37°C for at least 2 hours before use.

Day 0

~4 hours

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

Treatment

1. Determine the number of wells required to get 2.0 x 10^6 cells from the source hPSC 6-well plate.
2. Prepare Medium iN1 by mixing together the following components in a 15 ml conical tube.
    - Warm Medium N1, iROCK, and Solution D1 at room temperature for at least 1 hour protected from light.
    - The rest of Medium N1 should be stored at 4°C for later use.

	Required medium volume based on # of wells of a 6-well plate	
Reagents	1 well	2 wells
Medium N1	8.3 ml	9.4 ml
iROCK	8.3 µl	9.4 µl

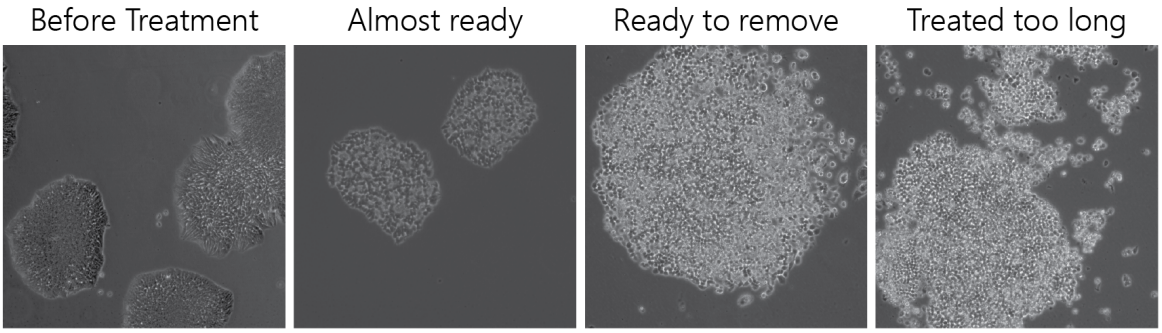
3. Referring to the table below, prepare the required volume of hPSC maintenance medium with iROCK in a new 15 ml conical tube. Mix well and allow to warm at room temperature for 20-30 minutes.

	Required volume per # of wells of a 6-well plate	
Reagents	1 well	2 wells
hPSC maintenance medium	1.5 ml	3.0 ml
iROCK	1.5 µl	3.0 µl

4. Aspirate old medium from hPSC culture and add 1.5 ml of hPSC maintenance medium with iROCK to each well.
5. Incubate the culture at 37°C, 5% CO_2 for 1 hour before harvesting cells.
    - This is to decrease cell death on Day 1 and minimize the loss of cells.

- During the incubation, start thawing QGA-SeV (undiluted) on ice and warming Solution D1 at room temperature.

6. Aspirate old medium from hPSC culture and add 2 ml PBS to each well being harvested.
7. Rock the plate 3 times, aspirate PBS from the culture, and add 300 µl of the cell dissociation reagent Solution D1 to each well.
    - Keep the rest of Solution D1 at 4°C for use on Day 4.
8. Incubate the culture plate at 37°C, 5% CO_2 for 5 minutes. If all the cells are not rounded under a microscope, continue to incubate at 37°C, 5% CO_2 in 1-2 minute increments (see images below).



9. Carefully pipet out Solution D1 from the culture and add 1 ml Medium iN1 to the well.
    - Follow steps 9-11 one well at a time if multiple wells are used.
10. Disperse the medium over the bottom surface of the well by pipetting 8-15 times to detach cells.
11. Using the same pipet tip, collect the cell suspension in a 1.5 ml tube.

IMPORTANT! In this protocol, users will treat hPSCs with QGA-SeV (undiluted) in a tube and then plate the cells onto 6 wells with 1 ml Medium iN1 (3.0×10^5 cells) per well. However, we recommend preparing a suspension of 6.6 ml to avoid insufficiency. First, QGA-SeV (undiluted) should be mixed with 340 µl of a dense cell suspension to increase the chance that QGA-SeV (undiluted) finds its host cells. After 10 minutes incubation at 33°C, the total volume will be brought up to 6.6 ml with Medium iN1. Cell count may vary based on cell health, the method, and instrument used for cell counting.

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12. Count cells and determine viability.
13. Take out the volume of the cell suspension needed for 6 wells and include 10% extra (a total of 1.98×10^6 cells to plate 0.3×10^6 cells in each of the 6 wells). Transfer the determined volume of the cell suspension into a 15 ml conical tube.
14. Bring the volume of the cell suspension up to 340 µl with Medium iN1.
    - If the volume of the cell suspension needed to get 1.98×10^6 cells exceeds 340 µl, centrifuge the required volume of cell suspension at 200 x g for 4 minutes, remove the supernatant, and resuspend the pellet into 340 µl Medium iN1.

IMPORTANT! Before adding QGA-SeV (undiluted), ensure that it is fully thawed. Do not centrifuge, vortex, or mix SeV with a pipettor; SeV is highly sensitive to physical stress.

15. Add all the contents of the QGA-SeV tube to the hPSCs and mix them by tapping with a finger 2-3 times. Cap the tube loosely to allow gas exchange.
16. Incubate the cell suspension at 33°C, 5% CO_2 for 10 minutes with intermittent mixing, by finger tapping, every 2 minutes.

Plating

1. Bring up the volume of cell suspension to 6.6 ml with Medium iN1 and mix 2-3 times with a serological pipet.
2. Aspirate PBS from only one coated well at a time and add 1 ml cell suspension to each well. Most of the PBS should be aspirated but not completely to prevent the coated wells from drying before adding the cell suspension. Likewise, the cell suspension should be added to the well immediately after PBS is removed. Handle one well after another.
3. Move the plate in 5 cycles of quick back-and-forth and side-to-side motions to evenly distribute treated cells in the cultures.

4. Incubate the culture plate at 33°C, 5% CO_2 overnight.

Day 1

< 1 hour

Medium Change

1. Prepare Medium A1 by mixing together the following components in a 50 ml conical tube.
    - Warm Medium N1 at room temperature for 20-30 minutes.
    - Thaw 4 vials of Component GA1 on ice for 20 minutes.
    - Thaw 4 vials of Component GA2, protected from light, at room temperature for 20 minutes.
    - The rest of Medium A1 should be stored at 4°C for its use on Day 2.

Reagents	Volume
Medium N1	30 ml
Component GA1	60 µl
Component GA2	60 µl

2. Pipet out the medium from each well, leaving a small volume behind to avoid the cells drying out, and add 1.5 ml Medium A1.
3. Incubate the culture plate at 33°C, 5% CO_2 overnight.

Day 2

< 1 hour

Medium Change and Temperature Shift

Note: This should be performed in the late afternoon.

1. Warm Medium A1 at room temperature for 20-30 minutes.
2. Pipet out the medium from each well, leaving a small volume behind to avoid the cells drying out, and add 1.5 ml Medium A1.
3. Incubate the culture plate at 37°C, 5% CO_2 overnight.

Day 3

< 1 hour

IMPORTANT! Following this protocol, users will encounter cell death in the infected cultures. It will be most noticeable the day after making the temperature shift. When monitoring the health of infected cultures, please refer to the images in the appendix showing the recovery trajectory of a typical hPSC culture subjected to QGA-SeV-mediated differentiation. It is optional, but recommended, to include the PBS wash if cell death/floating cells are observed.

Maintenance

1. Warm Medium A1 at room temperature for 20-30 minutes.
2. Pipet out the medium from each well, leaving a small volume behind to avoid the cells drying out, and add 1.5 ml Medium A1.
   *(Optional) Slowly add 1.5 ml PBS alongside the wall of each well to avoid lifting attached cells. Gently pipet out PBS before adding Medium A1.
3. Incubate the culture plate at 37°C, 5% CO_2 overnight.

Day 4

< 2 hours

Medium Change

1. Prepare ScienceCell Medium with 2% FBS by mixing together the following components in a 50 ml conical tube.
    - Warm Basal Medium, Astrocyte Growth Supplement (AGS), and Pen/Strep (P/S) from the ScienCell kit at room temperature for 1 hour away from light.
    - Thaw FBS from the ScienCell kit at room temperature for 1 hour.
    - Aliquot and store unused AGS and FBS at -20°C and the Basal Medium and P/S at 4°C.

Reagents	Volume
Basal Medium	80 ml
AGS	830 µl
P/S	830 µl
FBS	1.67 ml

2. Pipet out the old medium from each well , leaving a small volume behind to avoid the cells drying out.
3. Add 2.5 ml of ScienCell Medium with 2% FBS to each well, slowly along the wall of the well.
4. Incubate the cultures at 37°C, 5% CO_2 for 3 days.

Days 7 -16

< 1 hour

Maintenance

1. Warm ScienCell Medium with 2% FBS at room temperature for 30 minutes.
2. Pipet out 1.25 ml of the old medium from each well.
3. Add 1.25 ml of ScienCell Medium with 2% FBS to each well, slowly along the wall of the well.
4. Incubate the cultures at 37°C, 5% CO_2.
5. Repeat steps 1-4 every 2-3 days.

Day 17

~ 2 hours

New Plate Preparation

IMPORTANT! Cells can be plated on 6-well, 24-well, or 96-well plates depending on the desired format. This kit can accommodate replating to all wells of either a 6-well, a 24-well, or a 96-well plate. Refer to the tables at the bottom of this page for the recommended volumes. Please note that the volumes are per plate in Table A and per well in Table B. Surplus cells can be frozen following the instructions in Appendix B.

1. Aliquot the volume of Geltrex specified in Table A to a prechilled 15 ml conical tube and keep on ice.
2. Add Geltrex to wells according to Table B.
3. Incubate the plate at 37°C, 5% CO_2 for at least 1 hour or until cells are ready for plating. Alternatively, coating can be performed by incubating the plate at 4°C overnight.
4. Warm ScienCell Medium (without FBS) at room temperature for 30-40 minutes.
5. After the Geltrex incubation, aspirate most, but not all of, the supernatant and add ScienCell Medium (without FBS) in the volume specified in Table B.
6. Incubate the plate at 37°C, 5% CO_2 until cells are ready for plating.

Table A. Recommended volumes per plate for different plate formats.

Reagents	Required volume per plate		
	6-well plate	24-well plate	96-well plate
Geltrex	10 ml	8 ml	5.3 ml
ScienCell Medium (without FBS)	16.5 ml	20 ml	12 ml

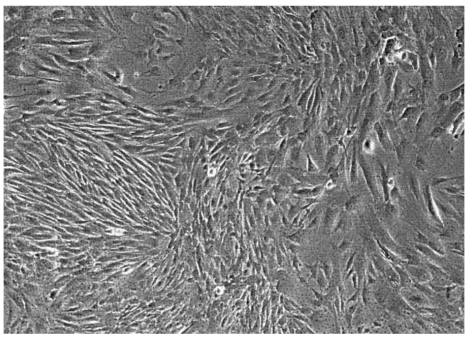
Table B. Recommended volumes per well for different plate formats.

Reagents	Required volume per well		
	6-well plate	24-well plate	96-well plate
Geltrex	1.5 ml	300 µl	50 µl
ScienCell Medium (without FBS)	1 ml	400 µl	34 µl

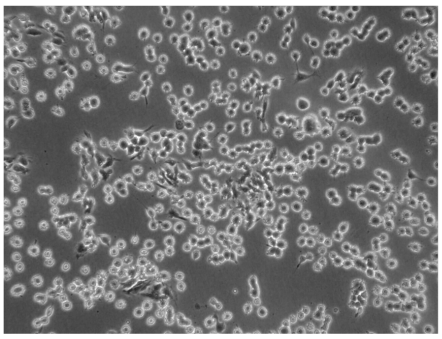
Passaging Cells

IMPORTANT! For the following steps, gently pipet and add solutions. Differentiating cells are delicate and should be handled with great care. Steps 2-10 below are critical. **Perform these steps one well at a time.** Refer to the images below to successfully manage cell treatment and dissociation.

Before Solution D1 Treatment



After Solution D1 Treatment



1. Warm Solution D1 at room temperature for at least 1 hour before use.
2. Pipet out the old medium from one well and add 1 ml PBS and gently rock the plate.
3. Pipet out the PBS from the well and add 300 µl Solution D1.
4. Rock the plate 3 times to spread the Solution D1 evenly.
5. Incubate the cultures at 37°C, 5% CO_2 for 5 - 10 minutes.
6. Gently pipet out Solution D1 from the well and add 1 ml ScienCell Medium **(without FBS)** to the well along the wall of the well.
7. Disperse the medium quickly over the bottom surface of the well by pipetting 6-8 times to detach cells using a P1000 pipettor.
8. Observe cells and/or cell aggregates floating in the well under a microscope. It is normal that 10-20% of cells remain attached to the well bottom after pipetting. Do not attempt to detach all of the cells remaining on the well bottom.
9. Collect 1 ml cell suspension from the well and transfer to a tube.
10. Repeat steps 2-9 for the rest of the wells.
11. Gently pipet the cell suspension up and down up to 5 times to break the cell aggregates. Excessive pipetting can damage the already-suspended neuronal cells.
12. Count cells and determine viability.
13. Prepare 0.5 x 10^6 viable cells/ml cell suspension using ScienCell Medium **(without FBS)** based on the table below.

a. If there are leftover cells, freeze the cells down by following instructions (beginning at step 2) in Appendix B after plating cell suspensions to the new plate. Keep the leftover cells on ice until freezing.

14. Add cell suspension to the center of each well. Since each well already has ScienCell Medium **(without FBS)**, the total volume of the medium in each well is indicated in the table below.

Note: The cell plating density recommended should result in confluent cultures in about two weeks. If users desire confluence in a shorter or longer period they should adjust the plating density accordingly. In addition, the density recommended may not be optimal for all hPSC cell lines as growth rates can vary depending on the hPSC cell line.

	Recommended amounts		
	6-well plate	**24-well plate**	**96-well plate**
Viable cells/well	2.5 x 10^5 cells	5 x 10^4 cells	8 x 10^3 cells
Required volume of cell suspension (0.5 x 10^6 viable cells/ml) ○ (Vol of cell suspension/well x # of wells) + 10% extra	3.3 ml	2.64 ml	1.6 ml
Volume of cell suspension/well	500 µl	100 µl	16 µl
Total volume/well ○ ScienCell Medium **(without FBS)** + cell suspension	1.5 ml	500 µl	50 µl

15. Move the plate in 5 cycles of quick back-and-forth and side-to-side motions to evenly distribute treated cells in the cultures.
16. Allow cells to attach by incubating the plate at room temperature for 10 minutes.
17. Incubate the cultures at 37°C, 5% CO_2 overnight.

Day 18

< 1 hour

Maintenance

1. Warm ScienCell Medium **(without FBS)** at room temperature for 30-40 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 **(without FBS)** to each well according to the table below.
4. Incubate the cultures at 37°C, 5% CO_2 for 2-3 days.

	Required volume per well		
Reagents	**6-well plate**	**24-well plate**	**96-well plate**
ScienCell Medium **(without FBS)**	2.5 ml	1 ml	0.25 ml

Days 20-27

< 1 hour

Maintenance

1. Warm ScienCell Medium **(without FBS)** at room temperature for 30-40 minutes.
2. Pipet out half of the volume of old medium from each well (see Table above).
3. Add an equal volume of fresh ScienCell Medium **(without FBS)** to each well.
4. Incubate the cultures at 37°C, 5% CO_2.
5. Repeat steps 2-4 every 2-3 days, making more ScienCell Medium as needed following the instructions above.

Day 28

Assay or Continuous Maturation

- CD44, S100β, GFAP, and ALDH1L1-positive cells can be detected on Day 28.
- For more mature astrocytes with increased expression of GFAP and ALDH1L1, we recommend culturing cells until Day 42. When cells approach confluence they can be passaged following the instructions on Day 17, though maintaining them at higher confluency results in higher GFAP expression.
- From Day 28, users may maintain differentiated astrocytes in the maintenance medium best suited for their needs, though we recommend ScienCell Astrocyte Medium without FBS.
- Cells can be frozen following the instructions in Appendix B.

Appendix A

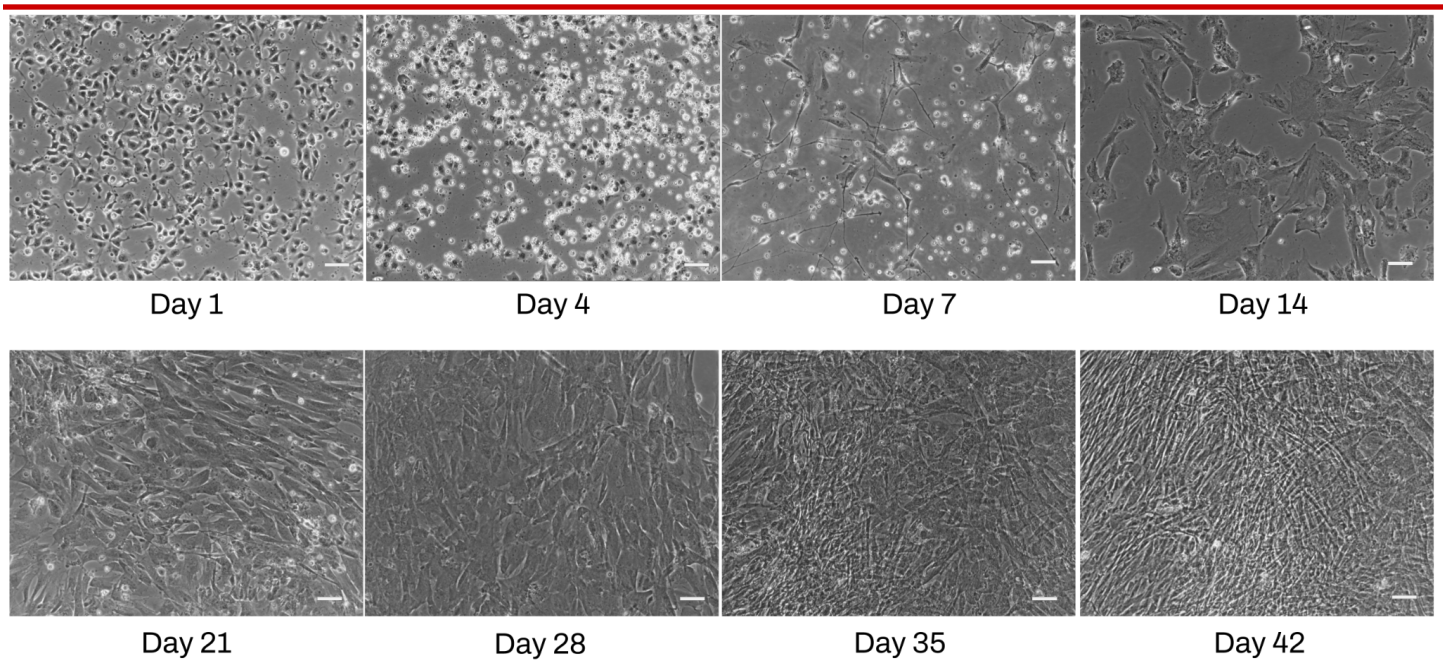


Figure 1. Representative images of Quick-Glia™ Astrocyte - SeV Kit (Small) cell cultures on days 1, 4, 7, 14, 21, 28, 35 and 42 post-differentiation (scale bar = 100 µm). User's cultures may display a slightly lower level of confluency on each day due to minor differences between small and large Quick-Glia™ Astrocyte - SeV Kit formats.

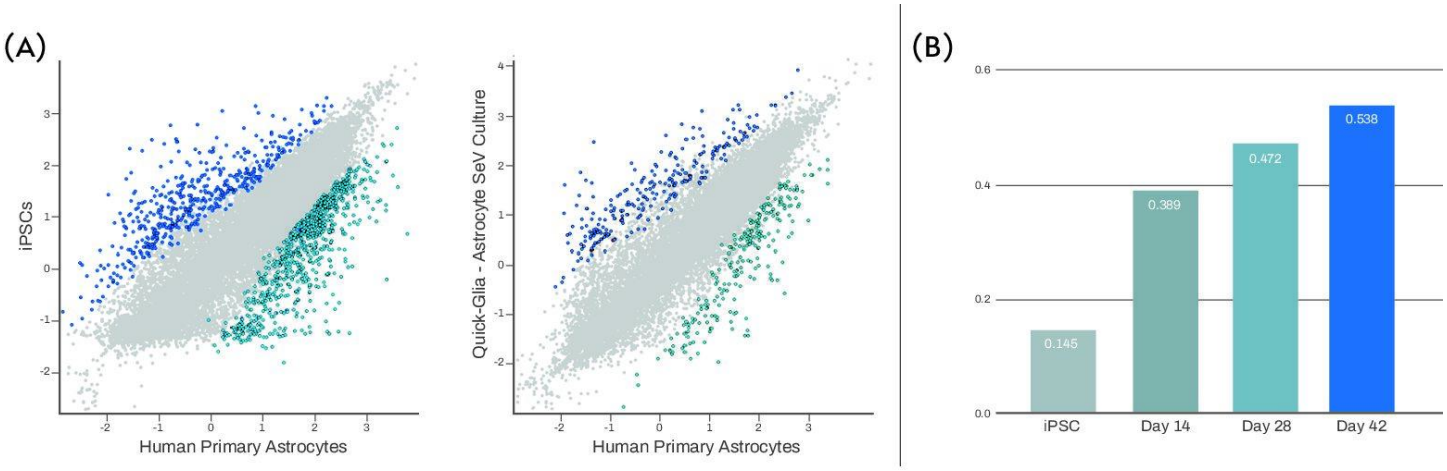


Figure 2. (A) Gene expression profiles of iPSCs and Quick-Glia™ - Astrocyte SeV Culture on day 28 were compared with the profile of human primary astrocytes and the results are shown as scatter plots. The horizontal axis indicates the expression levels of genes in human primary astrocytes purchased from ScienCell (Catalog Number: 1800-5), whereas the vertical axis indicates the expression levels of genes in iPSCs (left) and in Quick-Glia™ - Astrocyte SeV Culture on day 28 (right). The levels of gene expression are shown based on transcripts per million (TPM) in the log10 scale. Blue and green dots represent upregulated and downregulated genes (FDR<0.05), respectively, relative to their levels in human primary astrocytes. **(B)** Similarities of gene expression profiles of human iPSCs and Quick-Glia™ - Astrocyte SeV Culture on days 14, 28 and 42 to the profile of human primary astrocytes are shown as a bar chart. The vertical axis indicates Pearson correlation (r) based on median-subtracted logTPM.

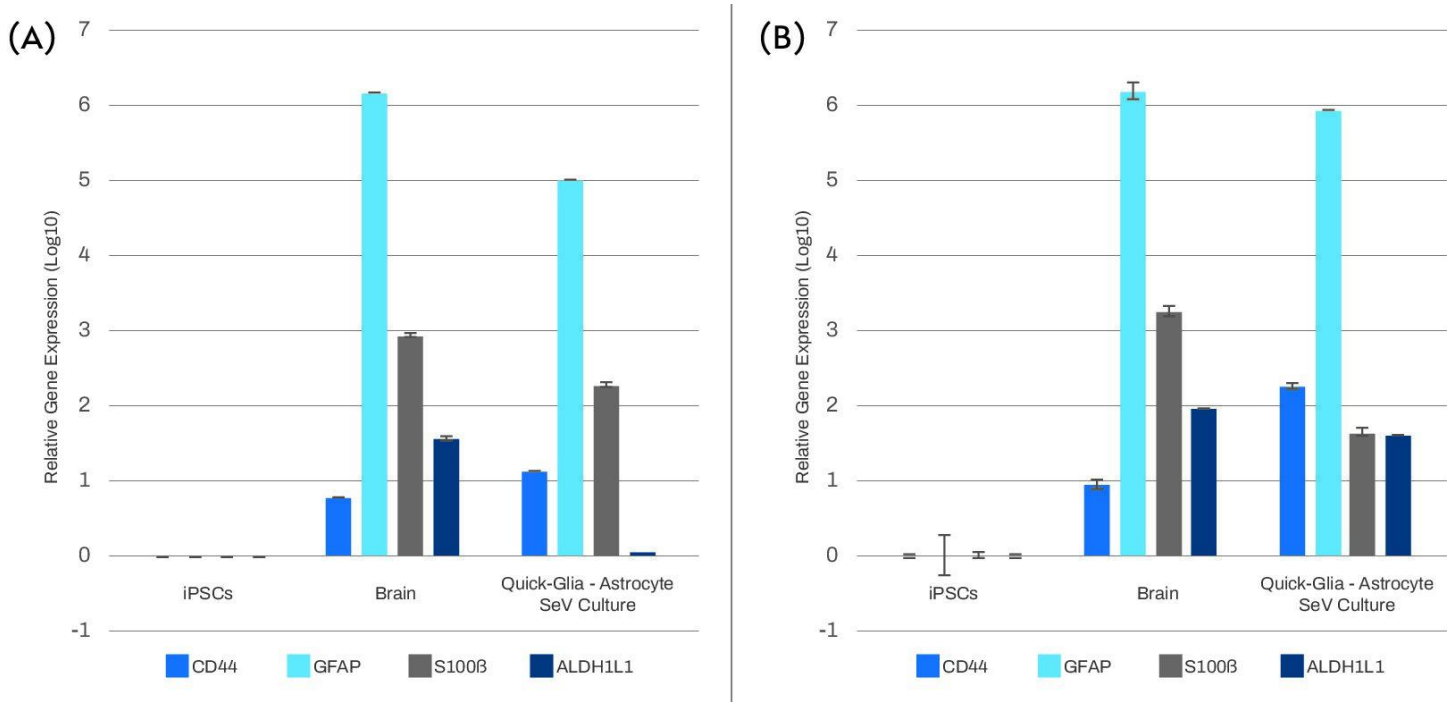


Figure 3. Real-time quantitative PCR analysis of expression levels of astrocyte-associated genes CD44, GFAP, S100β and ALDH1L1 were examined. Graphs show comparison of gene expression in Quick-Glia™ - Astrocyte SeV Culture on day 28 **(A)** and day 42 **(B)** with gene expression in human brain total RNA (TaKaRa, Catalog Number: 636530). The relative gene expression is normalized to phosphoglycerate kinase 1 (PGK1), and then calculated as a fold induction relative to undifferentiated hPSCs as a control. Error bars show standard deviation. Primers used are listed in Table 1.

Table 1. List of PCR primers used in Figure 3

Gene	Forward primer	Reverse Primer	Primer Concentration
CD44	CTGCCGCTTTGCAGGTGTA	CATTGTGGGCAAGGTGCTATT	250 nM
GFAP	ATCGAGAAGGTTCGCTTCCTG	TGTTGGCGGTGAGTTGATCG	250 nM
S100β	GGCTGGTCTCAAACTTCCTG	TCCACAACCTCCTGCTCTTT	250 nM
ALDH1L1	TCACAGAAGTCTAACCTGCC	AGTGACGGGTGATAGATGAT	250 nM
PGK1	GTATGCTGAGGCTGTCACTCG	CCTTCCAGGAGCTCCAAACTGG	250 nM

Appendix B

Freezing Cells Down

Note: After thawing frozen cells, approximately 80% of cells will be viable.

1. Follow steps 1-12 in the “Passaging cells” section of Day 17.
2. Determine the volume of the cell suspension and number of cryovials needed to freeze 1-4 x 10^6 cells per cryovial.
3. Centrifuge at 200 x g for 4 minutes.
4. While waiting for the centrifugation, label each cryovial. We recommend writing the name of the iPSC line used, the type of neurons, harvesting day and date, and the number of cells in the vial.
5. Aspirate the supernatant and resuspend the pellet with 0.5 ml/vial STEM-CELLBANKER.
6. Distribute 0.5 ml of the suspension to each cryovial.
7. Make sure that the caps are closed tightly and transfer the cryovials into a Mr. Frosty Freezing Container. Make sure that Mr. Frosty contains 250 ml isopropanol.
8. Loosely close the lid of Mr. Frosty with cryovials, put it into a -80°C freezer and leave it overnight or up to a few days.
9. Transfer the cryovials into a liquid nitrogen storage tank.
10. Follow the thawing instructions in the user guide for Quick-Glia™ Astrocyte - Human iPSC-derived Astrocytes, Catalog Number: AS-SeV-CW.