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

Kit Contents

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

Reagents	Volume	Storage
QGA-SeV (undiluted)*	105 µl	-80°C
Component N1	2 x 830 µl	-20°C or -80°C
Component GA1	4 x 16 µl	-20°C or -80°C
Component GA2	4 x 16 µl	-20°C or -80°C

***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.

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

Last revised: May 27, 2021

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 (100x)	ThermoFisher	35050061
Penicillin-Streptomycin	ThermoFisher	15140122
iMatrix-511 silk	Elixirgen Scientific	NI511S
TrypLE Select Enzyme (1X)*	ThermoFisher	12563011
0.02% EDTA in DPBS	Sigma-Aldrich	E8008-100ML
Geltrex hESC-Qualified, Ready-To-Use, Reduced Growth Factor Basement Membrane Matrix	ThermoFisher	A1569601
Phosphate-buffered saline (without Ca^{++} Mg^{++})	ThermoFisher	20012050
ROCK inhibitor Y27632	Selleckchem	S1049
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D8418
ScienCell Astrocyte Medium Kit: • Basal Medium • Astrocyte Growth Supplement • FBS • P/S	ScienCell Research Laboratories	1801
(Optional) STEM-CELLBANKER**	AMSBIO	11890

* Can be substituted with our Cell Dissociation Reagent (Solution D1), Catalog Number: CDR.

** This is only required if you intend to cryopreserve the cells after differentiation.

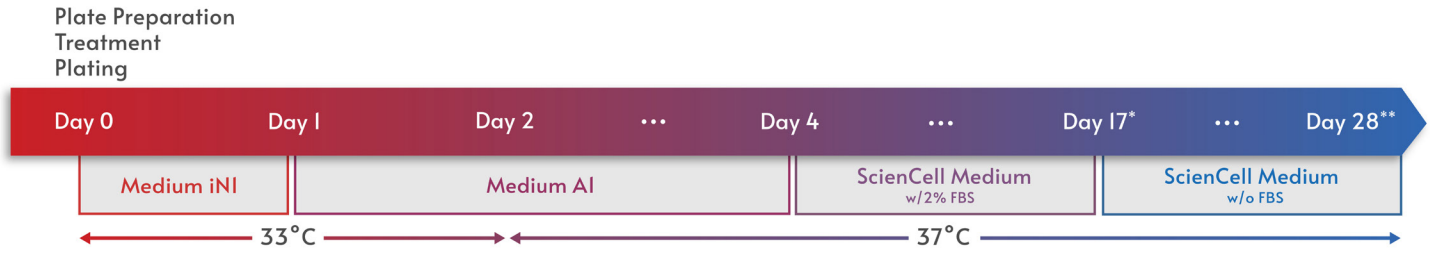
Source hPSC Culture Conditions

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, 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 1-2 35-mm culture dishes or 1-2 wells of a 6-well plate. If iMatrix-511 silk is routinely used as a coating substrate, prepare 1-2 culture dishes or wells precoated with 0.25 µg/cm² iMatrix-511 silk diluted in 2 ml chilled PBS 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.
- 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.



* Beginning at Day 17, if the cells are approaching confluence, users may choose to passage the cells or to cryopreserve them.

** 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.

*Can be substituted with our Cell Dissociation Reagent (Solution D1), Catalog Number: CDR.

Medium N1

1. Prepare Medium N1 using the reagents listed in the table below.
   - Warm all required reagents at room temperature for 1 hour.
2. Store Medium N1 for up to 2 weeks at 4°C. The leftover reagents can be discarded or saved for other uses.

Medium N1 Reagents	Volume
DMEM/F12	20.2 ml
Neurobasal Medium	20.2 ml
200 mM Glutamax (100x)	210 µl
Penicillin-Streptomycin (10000 units/ml; 100x)	420 µl
Component N1	1.26 ml

Day 0

~5 hr

This user guide assumes Day 0 is a Monday.

Plate Preparation

1. Prepare diluted iMatrix-511 silk by mixing together the components at the top of page 4 in a 15 ml conical tube.
   - Keep iMatrix-511 silk on ice.
   - Make sure chilled PBS is used for this mixture.

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

2. Add 2 ml diluted iMatrix-511 silk to each well of a new 6-well plate.
3. Incubate the plate at 37°C, 5% CO_2 for 2 hours (or 4°C overnight one day before Day 0).
4. Aspirate the supernatant from each well and add 2 ml PBS.
5. Incubate the plate at 37°C, 5% CO_2 until the hPSCs are ready for plating.

Treatment

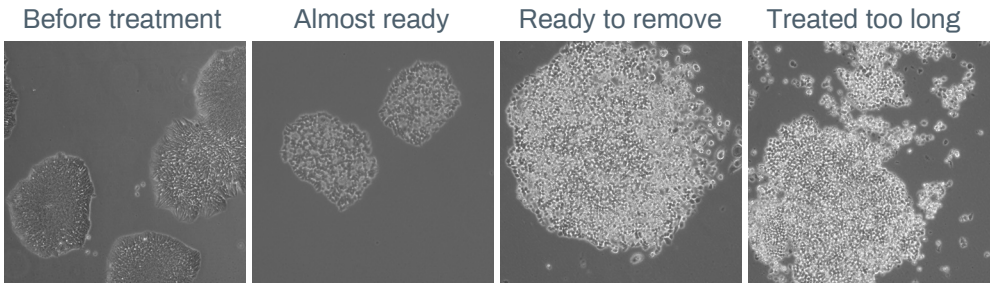
1. Determine the number of wells required to get 2.0 x 10^6 cells from the source hPSC 6-well plate.
2. Prepare Medium iN1 by mixing together the following components in a 15 ml conical tube.
    - Warm Medium N1 and iROCK at room temperature for 20-30 minutes.
    - The rest of Medium N1 should be stored at 4°C for later use.

Medium iN1 Reagents	Required volume per # of wells of a 6-well plate	
	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.

Reagents for hPSC Treatment	Required volume per # of wells of a 6-well plate	
	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 Solution D1 at room temperature.
6. Tap the Solution D1 tube 5 times with a finger and centrifuge at maximum speed for 1 minute.
7. Aspirate old medium from hPSC culture and add 2 ml PBS to each well being harvested.
8. Rock the dish/plate 3 times, aspirate PBS from the culture, and add 300 µl of Solution D1 to each well to begin cell dissociation treatment.
    - Keep the rest of Solution D1 at 4°C for its later use for optional passaging.
9. Incubate the culture at 37°C, 5% CO_2 for 5 minutes. If all the cells are not rounded under a microscope, incubate at 37°C, 5% CO_2 for up to 5 more minutes in 1-2 minute increments (see images below).



10. Carefully pipet out Solution D1 from the culture using a P1000 pipettor and add 1 ml Medium iN1 to each well.
    - Follow Steps 10-12 one well at a time if multiple wells are used.
11. Disperse the medium over the bottom surface of the well by pipetting 8-15 times to detach cells.
12. Using the same pipette 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 (0.3×10^6 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.

13. Count cells to determine the volume of cell suspension needed for 6 wells and include a 10% buffer (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. Adjust the volume 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.

14. Add 105 µl QGA-SeV (undiluted) to the hPSCs and mix them by tapping with finger 2-3 times. Cap the tube loosely to allow gas exchange.
15. 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 cultures at 33°C, 5% CO_2 overnight.

Day 1

 <1 hr

Medium Change

1. Prepare Medium A1 according to the table below in a fresh 15 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.
    - Keep the rest of Medium A1 at 4°C for its use on Day 2.

Medium A1 Reagents	Volume
Medium N1	30 ml
Component GA1	60 µl
Component GA2	60 µl

2. Pipet out the old 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 cultures at 33°C, 5% CO_2 overnight.

Day 2

<1 hr

Medium Change and Temperature Shift

1. Warm Medium A1 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, and add 1.5 ml Medium A1.
3. Incubate the cultures at 37°C, 5% CO_2 overnight.

Day 3

<1 hr

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 Appendix A showing the recovery trajectory of a typical hPSC culture subjected to QGA-SeV-mediated differentiation.

Maintenance

1. Warm Medium A1 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. Optional PBS wash (recommended for removal of cell debris):
    - Add 1.5 ml PBS to each well.
    - Pipet out the PBS wash from each well.
4. Add 1.5 ml Medium A1 to each well.
5. Incubate the cultures at 37°C, 5% CO_2 overnight.

Day 4

<2 hr

Medium Change

1. Prepare ScienCell Medium with 2% FBS using the reagents listed in the table below.
    - 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.

ScienCell Medium with 2% FBS	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 hr

Medium Change

1. Pipet out the old medium from each well, leaving a small volume behind to avoid the cells drying out.
2. Add 2.5 ml of ScienCell Medium with 2% FBS to each well, slowly along the wall of the well.
3. Incubate the cultures at 37°C, 5% CO_2.
4. Repeat steps 1-3 every 2-3 days.

Day 17

<2 hr

Medium Change

Beginning at Day 17, if the cells are approaching confluence, users may choose to passage the cells or to cryopreserve them by following instructions in Appendix B or C respectively.

1. Prepare ScienCell Medium **(without FBS)** using the reagents listed in the table below.
    - 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.

ScienCell Medium	Volume
Basal Medium	80 ml
AGS	820 µl
P/S	820 µl

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 **(without FBS)** to each well.
4. Incubate the cultures at 37°C, 5% CO_2 overnight.

Days 18-28

<1 hr

Medium Change

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 2.5 ml of ScienCell Medium **(without FBS)** to each well.
4. Incubate the cultures at 37°C, 5% CO_2.
5. Repeat steps 1-4 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. 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.

Appendix A

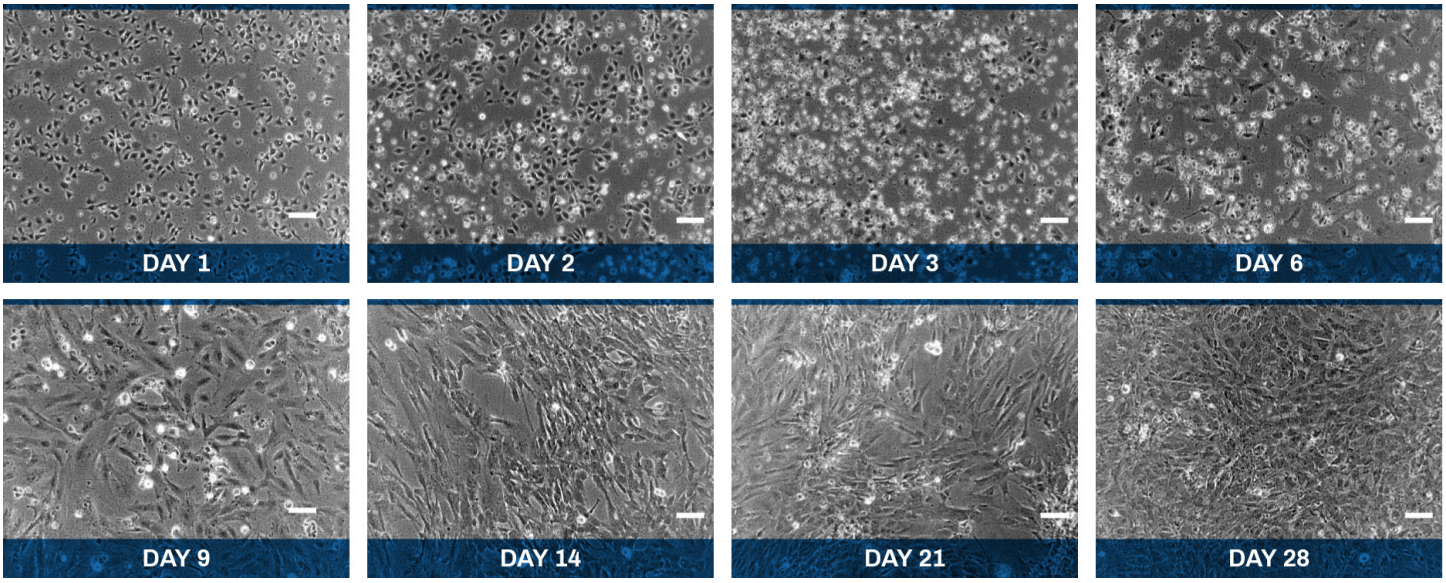


Figure 1. Representative images of Quick-Glia™ Astrocyte - SeV Kit (Small) cell cultures on days 1, 2, 3, 6, 9, 14, 21, and 28 post-differentiation (scale bar = 100 µm). User's cultures may display a slightly lower level of confluency on each day due to minor differences between small and large Quick-Glia™ Astrocyte - SeV Kit formats.

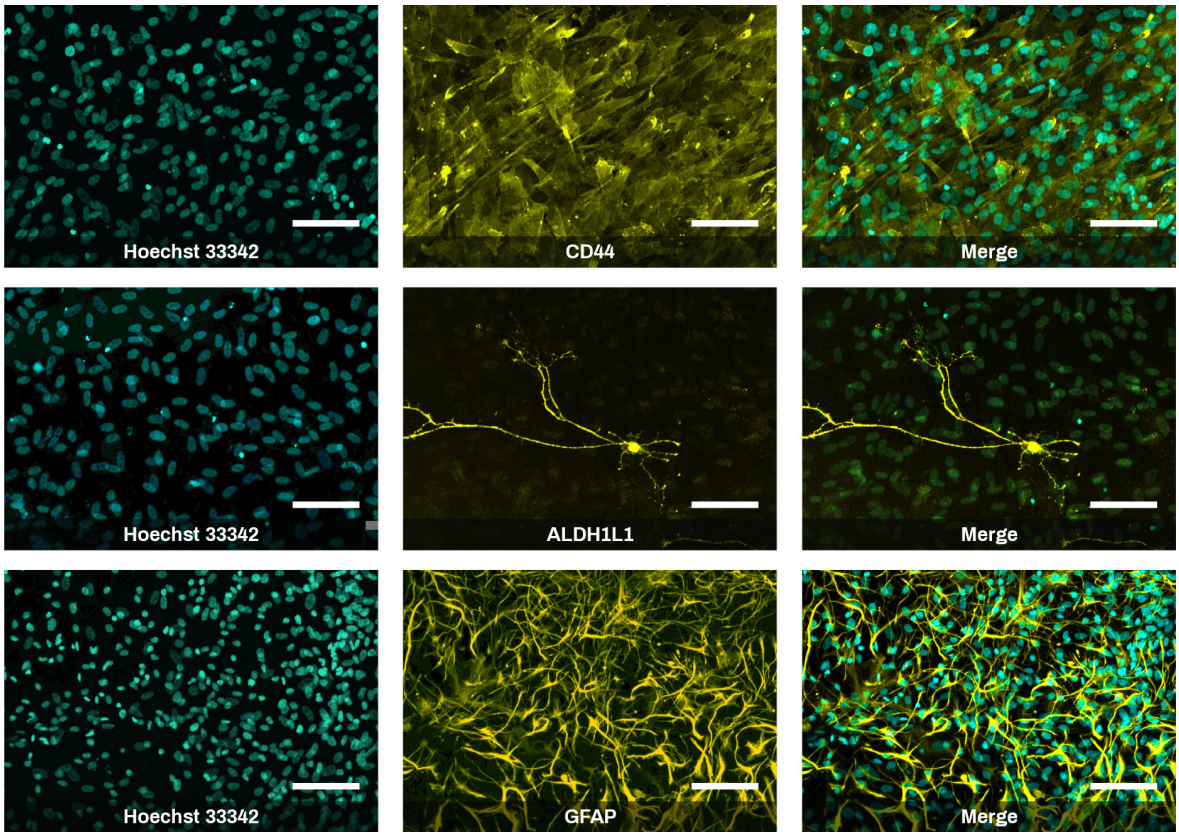


Figure 2. Immunofluorescent staining of Quick-Glia™ Astrocyte - SeV Culture on day 28 shows expression of astrocytic markers CD44, ALDH1L1, and GFAP. Nuclei are counterstained with Hoechst 33342 (cyan) (scale bar = 100 µm). All images are pseudo-colored. Primary antibodies used are Anti-CD44 (Cell Signaling Technology, Catalog Number: 3570, 1:400 dilution), Anti-ALDH1L1 (Abcam, Catalog Number: ab190298, 1:1000 dilution), Anti-GFAP (Cell Signaling Technology, Catalog Number: 3670, 1:300 dilution). Secondary antibodies used are Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, AlexaFluor Plus 488 (Invitrogen, Catalog Number: A32723, 1:500 dilution) and Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 (Invitrogen, Catalog Number: A-11037, 1:500 dilution).

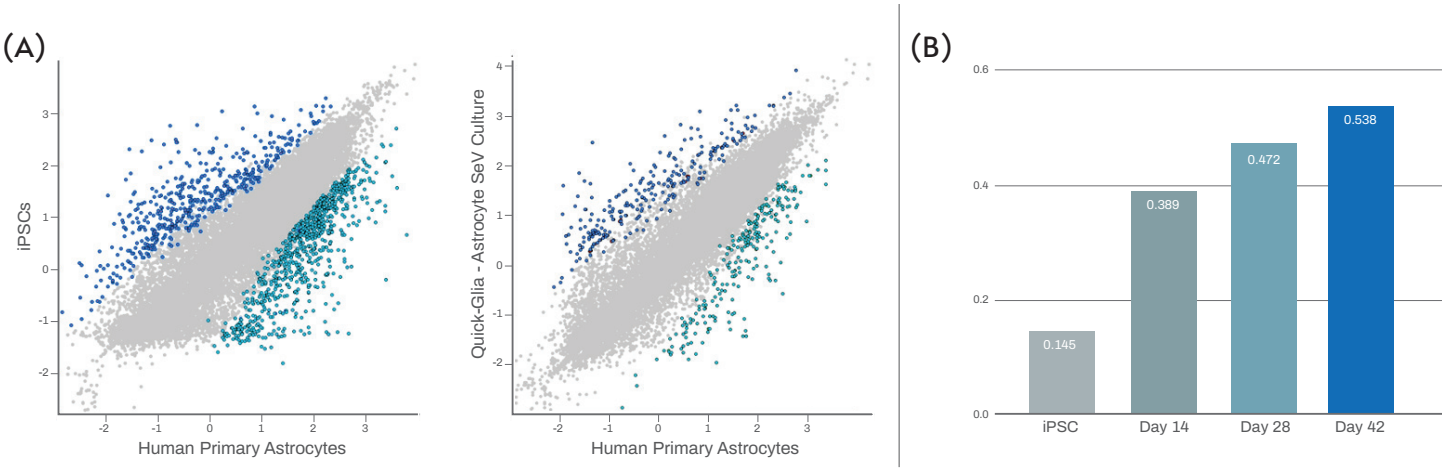


Figure 3. (A) Gene expression profiles of iPSCs and Quick-Glia™ - Astrocyte SeV Culture on day 28 were compared with the profile of human primary astrocytes and the results are shown as scatter plots. The horizontal axis indicates the expression levels of genes in human primary astrocytes purchased from 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.

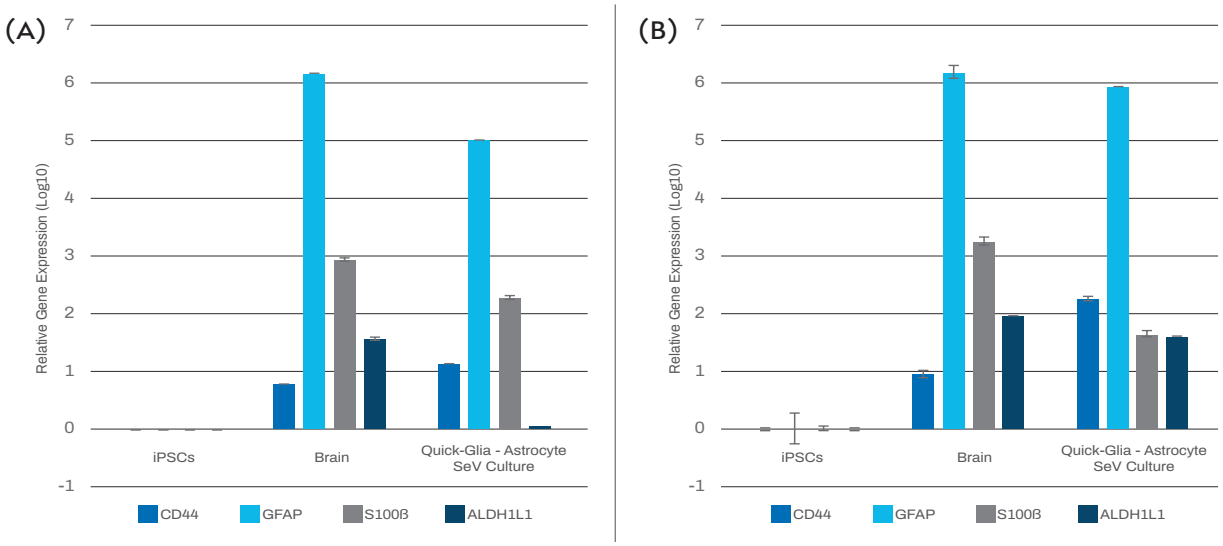


Figure 4. Real-time quantitative PCR analysis of expression levels of astrocyte-associated genes CD44, GFAP, S100β and ALDH1L1 were examined. Graphs show comparison of gene expression in Quick-Glia™ - Astrocyte SeV Culture on day 28 **(A)** and day 42 **(B)** with gene expression in human brain total 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 4

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

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 well in Table A and per plate in Table B. Surplus cells can be frozen following the instructions in Appendix C.

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	35 µl

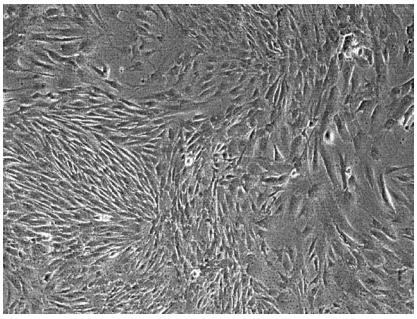
Harvesting Cells

IMPORTANT! For the following steps, gently pipet and add solutions. Differentiating cells are delicate and should be handled with great care.

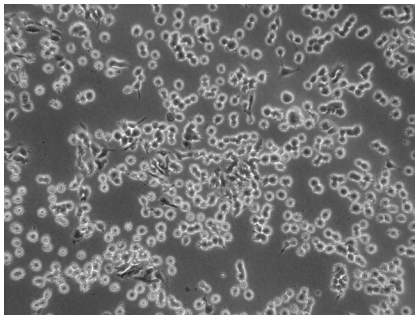
1. Warm Solution D1 at room temperature for at least 1 hour before use.
2. Tap the Solution D1 tube 5 times with a finger and centrifuge at maximum speed for 1 minute.
3. Working one well at a time, pipet out the old medium from each well using a P1000 pipettor and add 1 ml PBS and gently rock the plate.
4. Working one well at a time, pipet out the PBS from each well using a P1000 pipettor and add 300 µl Solution D1.
5. Rock the plate 3 times to spread the Solution D1 evenly.
6. Incubate the cultures at 37°C, 5% CO_2 for 3 minutes.
7. Working one well at a time, gently pipet out Solution D1 from each well using a P1000 pipettor and add 1 ml ScienCell Medium **(without FBS)** to each well along the wall of the well.

IMPORTANT! Steps 8-10 are critical. Perform these steps one well at a time. Refer to the images below to successfully manage cell treatment and dissociation.

Before Solution DI treatment



During Solution DI treatment



8. Working one well at a time, disperse the medium quickly over the bottom surface of the well by pipetting 6-8 times to detach cells using a P1000 pipettor.
9. 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. The clusters of cells are not supposed to be lifted. Do not attempt to detach all of the cells remaining on the well bottom.
10. Gently pipet the cell suspension up and down in the well up to 5 times to break the cell aggregates using a P1000 pipettor. Excessive pipetting can damage the already-suspended cells.
11. Collect all of the cell suspension from each well in a tube using the same P1000 pipette tip.
12. Count cells and determine viability.

Plating Cells

1. Prepare 1 x 10^6 viable cells/ml cell suspension using ScienCell Medium **(without FBS)** based on the table below.
    - If there are leftover cells, freeze the cells down by following instructions (beginning at step 2) in Appendix C after plating cell suspensions to the new plate. Keep the leftover cells on ice until freezing.
2. 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.

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

Reagents	Required volume per well		
	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
Reqired volume of cell suspension (1 x 10^6 viable cells/ml) • (Vol of cell suspension/well x # of wells) + 10% buffer	3.3 ml	2.64 ml	1.6 ml
Volume of cell suspension/well	500 µl	100 µl	15 µl
Total volume/well • ScienCell Medium **(without FBS)** + cell suspension	1.5 ml	500 µl	50 µl

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 cultures at 37°C, 5% CO_2 overnight.

After Passaging - Medium Change

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.

Reagent	Required volume per well		
	6-well plate	24-well plate	96-well plate
ScienCell Medium **(without FBS)**	2.5 ml	500 µl	100 µl

3. Incubate the cultures at 37°C, 5% CO_2.
4. Repeat steps 1-4 every 2-3 days, making more ScienCell Medium **(without FBS)** as needed following the instructions above.

Appendix C

Freezing Cells Down

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

1. Follow the instructions in the “Harvesting Cells” section of Appendix B.
2. Determine the volume of the cell suspension and number of cryovials needed to freeze 0.1 ~ 2 x 10^6 cells per cryovial.
3. Centrifuge at 200 x g for 4 min.
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.