User Guide

Quick-Glia™ Astrocyte - SeV Kit (Small)

Catalog Number: AS-SeV-S

Introduction

The Quick-Glia™ Astrocyte - SeV Kit (Small) 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 Sendai virus to produce highly pure populations of 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).

Scale: The Quick-Glia™ Astrocyte - SeV Kit (Small) contains a set of reagents for use with a total of 4 wells of a 24-well plate.

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*	120 µl	-80°C
Component N1	830 µl	-20°C or -80°C
Component GA1	16 µl	-20°C or -80°C
Component GA2	16 µl	-20°C or -80°C
Coating Material A	15.7 µl	-20°C or -80°C

This kit contains iMatrix-511 silk (Nippi, Inc.).

***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: April 21, 2021

Required Consumables

Item	Vendor	Catalog Number
24-well tissue-culture-treated polystyrene plate (e.g., Corning Costar Flat Bottom Cell Culture Plates)	Fisher Scientific	07-200-740
DMEM/F12	ThermoFisher	21331020
Neurobasal Medium	ThermoFisher	21103049
Glutamax (100x)	ThermoFisher	35050061
Penicillin-Streptomycin	ThermoFisher	15140122
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	D8418
ScienCell Astrocyte Medium Kit: • Basal Medium • Astrocyte Growth Supplement • FBS • P/S	ScienCell Research Laboratories	1801

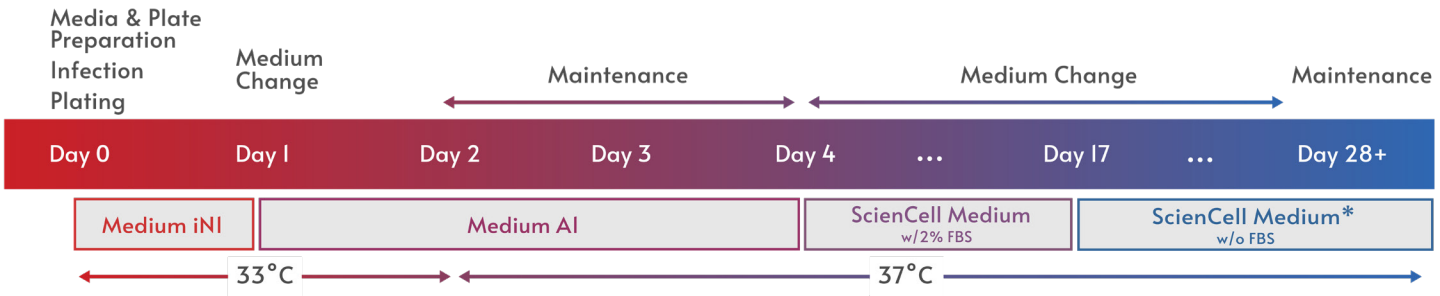
* Can be substituted with our Cell Dissociation Reagent (Solution D1), Catalog Number: CDR.

Source hPSC Culture Conditions

The Quick-Glia™ Astrocyte - SeV Kit (Small) 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 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 one culture dish or well 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.

Workflow



*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 is hereafter referred to as iROCK and can be stored at -20°C.

0.5X TrypLE Select with EDTA (Solution D1)*

1. Mix 1 ml TrypLE Select Enzyme (1X) with 1 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 N

1. Prepare Medium N1 using the reagents listed in the table below.
    - Warm all required reagents at room temperature for 1 hour.

Medium N1 Reagents	Volume
DMEM/F12	4.8 ml
Neurobasal Medium	4.8 ml
200 mM Glutamax (100x)	50 µl
Penicillin-Streptomycin (10000 units/ml; 100x)	100 µl
Component N1	300 µl

Day 0

~4 hr

Plate Preparation

1. Prepare diluted Coating Material A by mixing together the following components in a 15 ml conical tube.
    - Thaw Coating Material A on ice for 20-30 minutes (or at 4°C overnight one day before Day 0).
    - Make sure chilled PBS is used for this mixture.

Diluted Coating Material A Reagents	Volume
Coating Material A	6.6 µl
Chilled PBS	2 ml

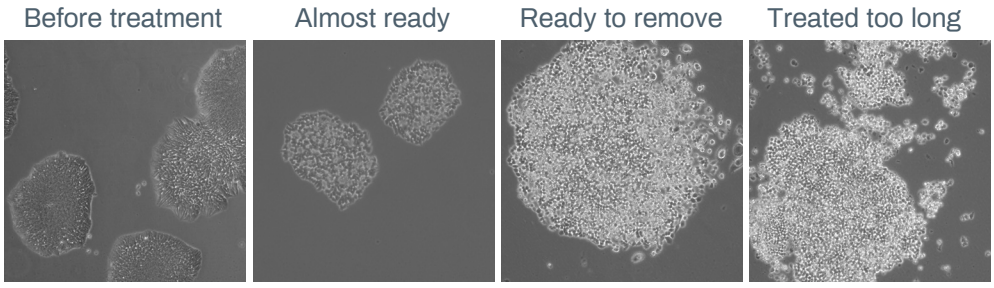
2. Add 400 µl diluted Coating Material A to each new well of 4 wells.
3. Incubate the plate at 37°C, 5% CO_2 for 2 hours (or 4°C overnight one day before Day 0).
4. Aspirate the supernatant from each well and add 500 µl PBS.
5. Incubate the plate at 37°C, 5% CO_2 until the hPSCs are ready for plating.

Treatment

1. 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.
    - The rest of Medium N1 should be stored at 4°C for later use.

Medium iN1 Reagents	Volume
Medium N1	3.5 ml
iROCK	3.5 µl

2. Thaw QGA-SeV on ice.
3. Tap the Solution D1 tube 5 times with a finger and centrifuge at maximum speed for 1 minute.
4. Aspirate old medium from hPSC culture and add 2 ml PBS.
5. Rock the plate 3 times, aspirate PBS from the culture, and add 300 µl of the cell dissociation reagent Solution D1. Keep the rest of Solution D1 at 4°C for use on Day 3.
6. 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).



7. Carefully pipet out Solution D1 from the culture using a P1000 pipettor and add 1 ml Medium iN1 to the well.
8. Disperse the medium over the bottom surface of the well by pipetting 8-15 times to detach cells.
9. 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 in a tube and then plate the cells onto 4 wells with 500 µl Medium iN1 (1.0×10^5 cells) per well. However, we recommend preparing a suspension of 2.2 ml to avoid insufficiency. First, QGA-SeV should be mixed with 110 µl of a dense cell suspension to increase the chance that QGA-SeV finds its host cells. After 10 minutes incubation at 33°C, the total volume will be brought up to 2.2 ml with Medium iN1. Cell count may vary based on cell health, the method, and instrument used for cell counting.

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10. Count cells to determine the volume of cell suspension needed for 4 wells and include a 10% buffer (a total of 4.4×10^5 cells to plate 1.0×10^5 cells in each of the 4 wells). Transfer the determined volume of the cell suspension into a 15 ml conical tube. Adjust the volume to 110 µl with Medium iN1. If the volume of the cell suspension needed to get 4.4×10^5 cells exceeds 110 µl, centrifuge the required volume of cell suspension at 200 x g for 4 minutes, remove the supernatant, and resuspend the pellet into 110 µl Medium iN1.

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

11. Add 110 µl QGA-SeV to the hPSCs and mix them by tapping with finger 2-3 times. Cap the tube loosely to allow gas exchange.
12. 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 2.2 ml with Medium iN1.
2. Aspirate PBS from only one coated well at a time and add 500 µl cell suspension to the 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 by according to the table below in a fresh 15 ml conical tube.
    - Warm Medium N1 at room temperature for 20-30 minutes.
    - Thaw Component GA1 on ice for 20 minutes.
    - Thaw Component GA2, protected from light, at room temperature for 20 minutes.
    - Keep the rest Medium A1 at 4°C for its use on Day 2.

Medium A1 Reagents	Volume
Medium N1	7 ml
Component GA1	14 µl
Component GA2	14 µl

2. Pipet out the old medium from each well using a P1000 pipettor and add 500 µl Medium A1.
3. Incubate the cultures at 33°C, 5% CO_2 overnight.

<1 hr

Day 2

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 using a P1000 pipettor, leaving a small volume behind to avoid the cells drying out, and add 500 µl Medium A1.
3. Incubate the cultures at 37°C, 5% CO_2 overnight.

<1 hr

Day 3

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.

Maintenance

1. Warm Medium A1 at room temperature for 20-30 minutes.
2. Pipet out the old medium from each well using a P1000 pipettor, leaving a small volume behind to avoid the cells drying out.
3. Optional PBS wash (recommended for removal of cell debris):
    - Add 500 µl PBS to each well using a P1000 pipettor.
    - Pipet out the PBS wash from each well using a P1000 pipettor.
4. Add 500 µl Medium A1 to each well using a P1000 pipettor.
5. Incubate the cultures at 37°C, 5% CO_2 overnight.

Days 4-16

 <2 hr

Medium Change

1. Prepare ScienCell Medium
    - 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.
    - Aliquot the Basal Medium in 49 ml aliquots.
    - Aliquot the AGS in 0.5 ml aliquots.
    - Store unused AGS aliquots at -20°C, store Basal Medium and the P/S at 4°C.

ScienCell Medium	Volume
Basal Medium	49 ml
AGS	500 µl
P/S	500 µl

2. Prepare ScienCell Medium with 2% FBS
    - Thaw FBS from the ScienCell kit at room temperature for 1 hour.
    - Aliquot FBS in 0.55 ml aliquots and store at -20°C.
    - The rest of ScienCell Medium should be stored at 4°C for up to 1 month.
    - Store unused AGS aliquots at -20°C, store Basal Medium and the P/S at 4°C.

ScienCell Medium with 2% FBS	Volume
ScienCell Medium (Basal + AGS + P/S)	30 ml
FBS	600 µl

3. Pipet out the old medium from each well using a P1000 pipettor, leaving a small volume behind to avoid the cells drying out.
4. Add 1 ml of ScienCell Medium with 2% FBS to each well using a 5 ml pipettor, slowly along the wall of the well.
5. Incubate the cultures at 37°C, 5% CO_2 for 2-3 days.
6. Repeat steps 3-5 every 2-3 days.

Days 17-28

 <1 hr

Medium Change - FBS Free Medium

1. Warm ScienCell Medium **(without FBS)** at room temperature for 30-40 minutes.
2. Pipet out the old medium from each well using a P1000 pipettor, leaving a small volume behind to avoid the cells drying out.
3. Add 1 ml of ScienCell Medium **(without FBS)** to each well using a 5 ml pipettor.
4. Incubate the cultures at 37°C, 5% CO_2 for 2-3 days.
5. Repeat steps 3-5 every 2-3 days, making more ScienCell Medium as needed following the instructions above.

Day 28

Assay or Continuous Maturation

CD44, S100β, and GFAP-positive cells can be detected on Day 28. For more mature astrocytes (ALDH1L1-positive cells), 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 Media. Differentiation into astrocytes can be confirmed with CD44, S100b, GFAP and ALDH1L1.

Appendix

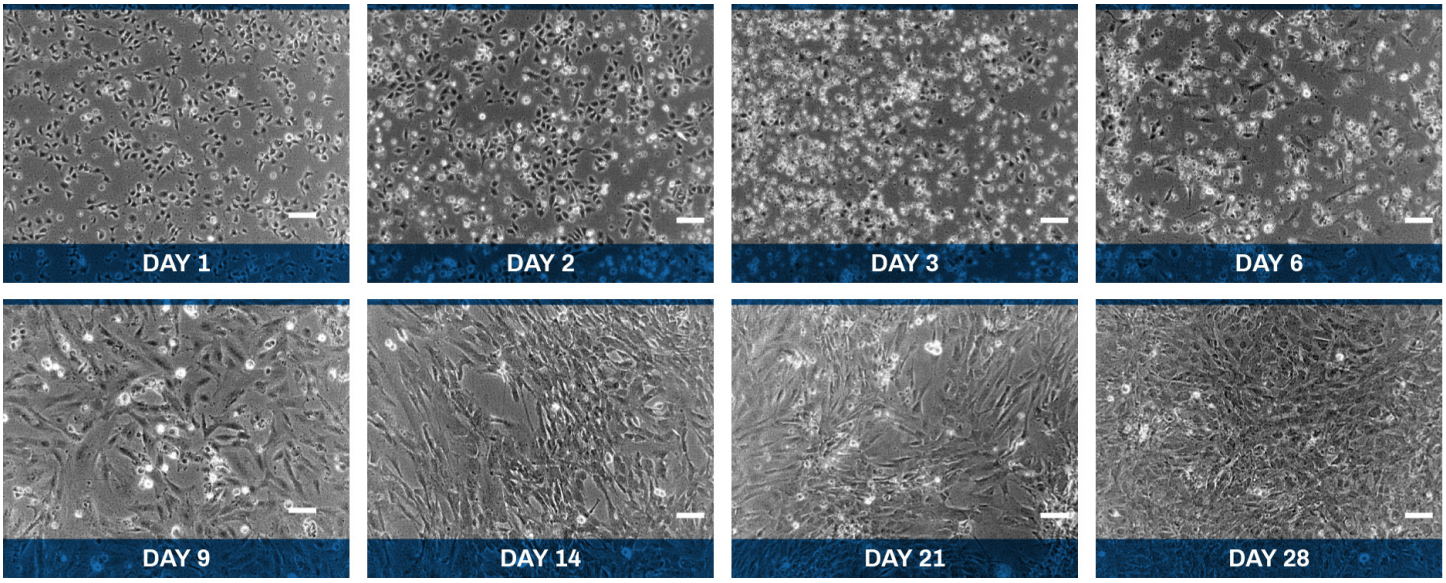

Figure 1. Representative images of Quick-Glia™ Astrocyte - SeV Kit cell cultures on days 1, 2, 3, 6, 9, 14, 21, and 28 post-differentiation (scale bar = 100 µm).

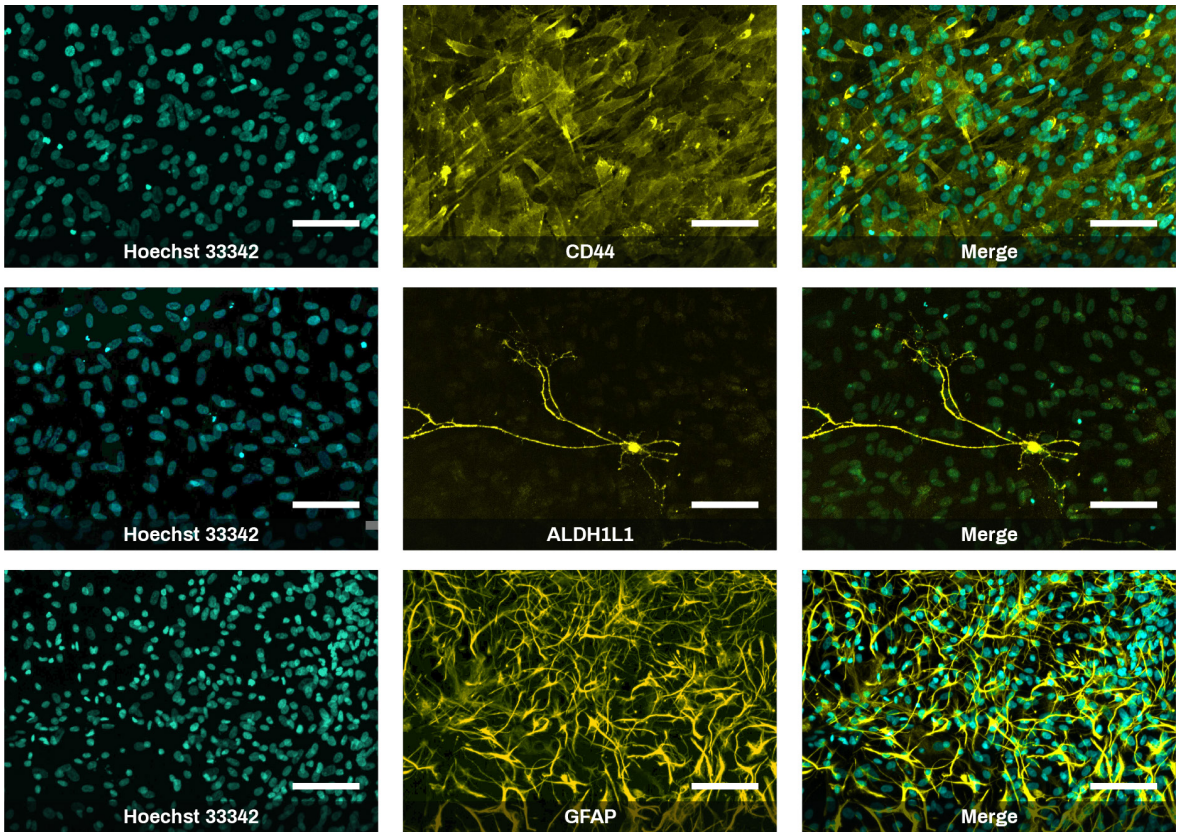

Figure 2. Immunofluorescent staining of Quick-Glia™ Astrocyte - SeV Culture on day 28 shows expression of astrocytic markers CD44, ALDH1L1, and GFAP. Nuclei are counterstained with Hoechst 33342 (cyan) (scale bar = 100 µm). All images are pseudo-colored. Primary antibodies used are Anti-CD44 (Cell Signaling 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).

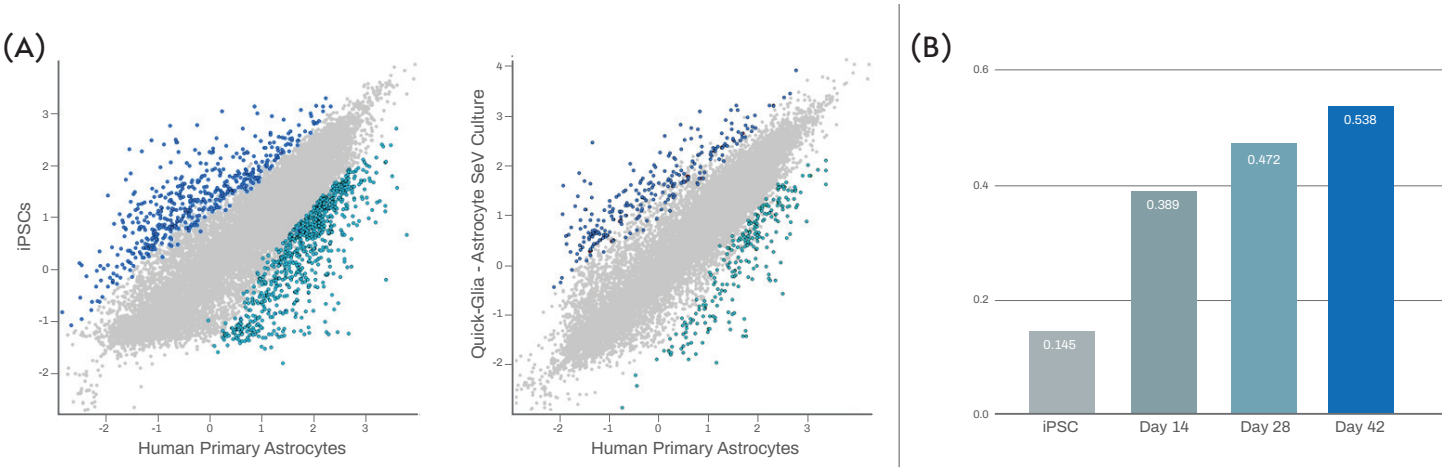

Figure 3. (A) Gene expression profiles of iPSCs and Quick-Glia™ - Astrocyte SeV Culture on day 28 were compared with the profile of human primary astrocytes and the results are shown as scatter plots. The horizontal axis indicates the expression levels of genes in human primary astrocytes purchased from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

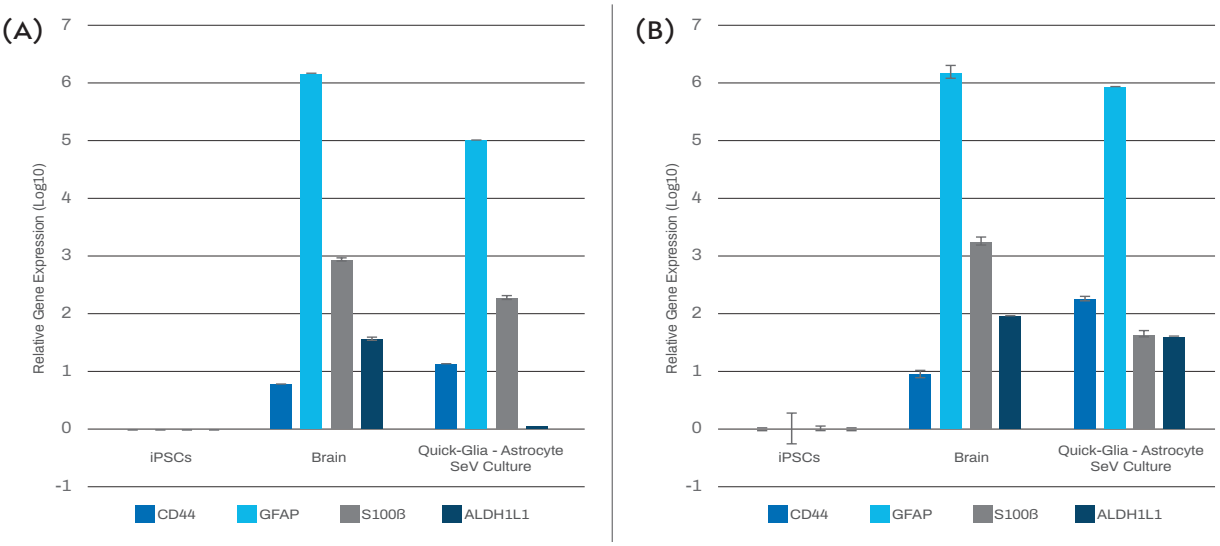

Figure 4. Real-time quantitative PCR analysis of expression levels of astrocyte-associated genes CD44, GFAP, S100β and ALDH1L1 were examined. Graphs show comparison of Quick-Glia™ - Astrocyte SeV Culture on day 28 **(A)** and day 42 **(B)** with human brain total RNA (TaKaRa, Catalog Number: 636530). 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