User Guide

ELIXIRGEN SCIENTIFIC

Quick-Glia™ Astrocyte - Human iPSC-derived Astrocytes

Catalog Numbers: AS-SeV-CW50065, AS-SeV-CW10149, AS-SeV-CW20300, AS-SeV-CW50023, AS-SeV-CW70067, AS-SeV-CW50025, AS-SeV-CW50113, AS-SeV-CW50114, AS-SeV-CW50115, AS-SeV-CW50137, AS-SeV-CW50147, AS-SeV-CW60130, AS-SeV-CW60231, AS-SeV-CW60236, AS-SeV-CW20026, AS-SeV-CW20090, or AS-SeV-CW10130

Introduction

Elixirgen Scientific's 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: Quick-Glia™ Astrocyte - Human iPSC-derived astrocytes are available in two sizes: (Small) 1 million viable cryopreserved cells and (Large) 5 x 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 4 wells of a 6-well plate (2.5 x 10^5 cells/well), 20 wells of a 24-well plate (5 x 10^4 cells/well), or 125 wells of a 96-well plate (8 x 10^3 cells/well).

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

Kit 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 (Small Size)	Amount (Large Size)	Storage
Cryopreserved cells	>1 million viable cells, (1 vial, 500 µl)	5 x >1 million viable cells, (5 vials, 5 x 500 µl)	Liquid nitrogen
Component P	2 x 14 µl	10 x 14 µl	-20°C

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: October 12, 2021

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., Corning Costar Flat Bottom Cell Culture Plates)	Fisher Scientific	12-566-70
Geltrex hESC-Qualified, Ready-To-Use, Reduced Growth Factor Basement Membrane Matrix	ThermoFisher	A1569601
ScienCell Astrocyte Medium Kit: • Basal Medium • Astrocyte Growth Supplement • FBS • P/S	ScienCell Research Laboratories	1801
(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

* 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.

Workflow

* 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 without the addition of Component P).

Media 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 at room temperature for 1 hour away from light.
    - Aliquot and store unused AGS at -20°C and the Basal Medium and P/S at 4°C.

Sciencell Medium (without FBS)	Volume
Basal Medium	59 ml
AGS	600 µl
P/S	600 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.

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.

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 5-10 x 10^3 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	2.5 x 10^5 cells	5 x 10^4 cells	8 x 10^3 cells

The cell plating densities recommended above 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 above may not be optimal for all hPSC cell lines as growth rates can vary depending on the hPSC cell line.

Day 0

Plate Preparation

IMPORTANT! Cells can be plated in 6-well, 24-well, and 96-well plates depending on the desired format. Refer to the table below 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 a 10% buffer. 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 using the volume of ScienCell Medium indicated in the table calculated for the number of wells in use plus 1.1 ml for resuspension (i.e., 4 wells of a 6-well plate needs 5.5 ml ScienCell Medium (1.1 x 4 + 1.1), 20 wells of a 24-well plate needs 9.9 ml ScienCell Medium (0.44 x 20 + 1.1) for this step).
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	Required 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. Warm ScienCell Medium at room temperature for 30-40 minutes.
2. Take out the vial of frozen cells from the liquid nitrogen storage tank.
3. Incubate the cryovial in a water bath set at 37°C (do not submerge the cap) until most of the content is thawed but a small ice crystal remains (~2 minutes).
4. Wipe the vial with a dry paper towel. Spray the vial with 70% ethanol and place it inside a biosafety cabinet.
5. Transfer 4.5 ml room temperature ScienCell Medium to a new 15 ml conical tube.
6. Set a P1000 pipettor to 1 ml but take approximately 500 µl ScienCell Medium from the 15 ml conical and add it to the cryovial dropwise at 1 drop per 1-2 seconds.
    - **IMPORTANT!** Use the same pipette tip for Steps 6-10.
7. Gently pipet the cell suspension up and down once.
8. Gently transfer all of the cell suspension to the 15 ml conical tube prepared in Step 5.
9. 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.

10. Mix the contents in the conical tube by gently pipetting cell suspension up and down 3 times.
11. Centrifuge the cell suspension in the 15 ml conical tube at 200 x g for 4 minutes.
12. 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.
13. Tap the side of the conical tube up to 10 times to break up the cell pellet.
14. 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 cells to determine the volume of cell suspension needed for chosen number of wells and include a 10% buffer for cell number and volume (e.g., for a 24-well plate scenario, a total of 1.1×10^6 cells to plate 5×10^4 cells in each of the 20 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	2.5×10^5 cells	5×10^4 cells	8×10^3 cells
Required total volume of cell suspension • (Volume of cell suspension per well) + 10% buffer	2.2 ml	2.2 ml	2.06 ml
Volume of cell suspension distributed per well	500 µl	100 µl	15 µl
Total volume per well • ScienCell Medium + 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 cells in the cultures.
4. Incubate the cultures at 37°C, 5% CO_2 overnight.

Day 1

Medium Change

1. Prepare ScienCell Medium (P) using the reagents listed in the table below.
    - Warm ScienCell Medium at room temperature for 30-40 minutes.
    - Thaw 2 vials of Component P at room temperature for 20-30 minutes.

ScienCell Medium (P)	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.

Reagent	Required volume per well		
	6-well plate	24-well plate	96-well plate
ScienCell Medium (P)	2.5 ml	500 µl	100 µl

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

Days 3-6

<1 hr

Maintenance

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

Reagent	Required volume per well		
	6-well plate	24-well plate	96-well plate
ScienCell Medium (P)	2.5 ml	500 µl	100 µl

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

Day 7

Note: If the cells are approaching confluence, users may choose to passage the cells or to cryopreserve them by following instructions in the Appendices.

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).

Appendix A

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 on 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 at room temperature for 30-40 minutes.
5. After the Geltrex incubation, aspirate most, but not all of, the supernatant and add ScienCell Medium 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	Recommended volume per plate		
	6-well plate	24-well plate	96-well plate
Geltrex	10 ml	8 ml	5.3 ml
ScienCell Medium	15.2 ml	13 ml	12 ml

Table B. Recommended volumes per well for different plate formats

Reagents	Recommended volume per well		
	6-well plate	24-well plate	96-well plate
Geltrex	1.5 ml	300 µl	50 µl
ScienCell Medium	1 ml	400 µl	35 µl

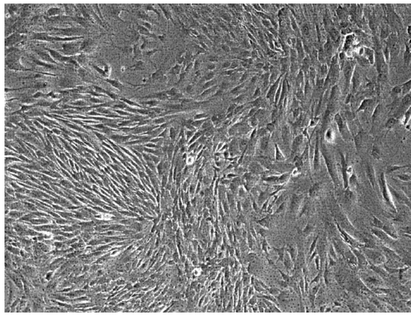
Harvesting Cells

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

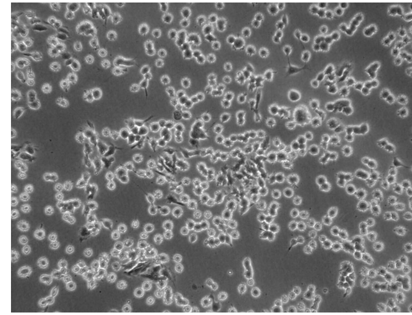
1. Warm Solution D1 at room temperature for at least 1 hour before use.
2. Tap the Solution D1 tube 5 times with a finger and centrifuge at maximum speed for 1 minute.
3. Working one well at a time, pipet out the old medium from each well using a P1000 pipettor and add 1 ml PBS and gently rock the plate.
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 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. 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 based on the table below.
    - 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.
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.

Note: 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.

	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
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 + 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 at room temperature for 30-40 minutes.

Reagent	Required volume per well		
	6-well plate	24-well plate	96-well plate
ScienCell Medium	2.5 ml	500 µl	100 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 to each well according to the table below.
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.

Appendix B

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 A.
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 this user guide.