User Guide

Quick-Neuron™ GABAergic - Human iPSC-derived Neurons

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

Introduction

Elixirgen Scientific's proprietary transcription factor-based technology allows rapid and reproducible differentiation of human iPSCs into neurons without sacrificing the purity of the cells. Our Quick-Neuron™ GABAergic - Human iPSC-derived Neurons display typical neurite outgrowth and express a variety of neuronal markers, such as the pan-neuronal marker tubulin beta 3 class III (TUBB3) and the GABAergic marker glutamic acid decarboxylase (GAD67). When handled and maintained according to the instructions in this user guide, GABAergic neurons are viable long-term and are suitable for a variety of characterization and neurotoxicity assays.

Scale: Quick-Neuron™ GABAergic - Human iPSC-derived Neurons are available in two sizes: (Small) 1 million viable cryopreserved cells and (Large) 5x 1 million viable cryopreserved cells. The instructions outlined in this user guide are for seeding 1 million viable cells into 10 wells of a 24-well plate (1 x 10^5 cells/well or 0.53 x 10^5 cells/cm^2).

Related Products: Quick-Neuron™ GABAergic - mRNA Kit, Catalog Number: GA-mRNA
Quick-Neuron™ GABAergic - SeV Kit, Catalog Number: GA-SeV
Quick-Neuron™ GABAergic - Maintenance Medium, Catalog Number: GA-MM

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 (LargeSize)	Storage
Cryopreserved cells	>1 million viable cells, (1 vial, 500 µl)	5x >1 million viable cells, (5 vials, 5x 500 µl)	Liquid nitrogen
Component N	2 x 840 µl	10x 840 µl	-20°C or -80°C
Component G1	2 x 16 µl	10 x 16 µl	-20°C or -80°C
Component G2	16 µl	5 x 16 µl	-20°C or -80°C
Component P	14 µl	5 x 14 µl	-20°C or -80°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 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).

Last revised: May 9, 2022

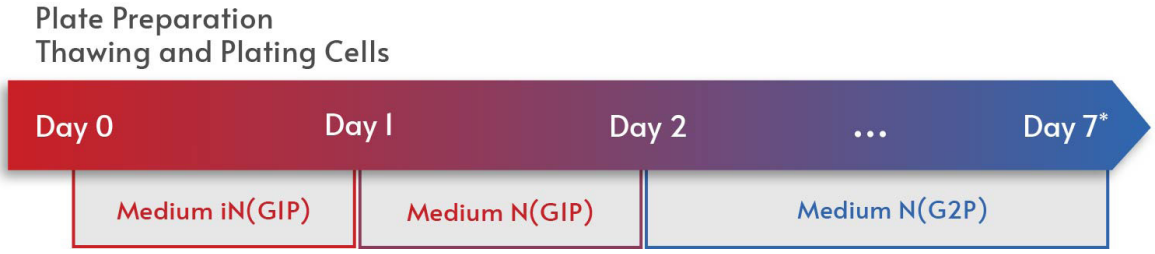
Required Consumables

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
0.01% Poly-L-Ornithine*	Sigma-Aldrich	P4957-50ML
Extracellular Matrix such as* • Laminin Mouse Protein, Natural, or • Geltrex Basement Membrane Matrix	ThermoFisher	 23017015 or A15696-01
Phosphate-buffered saline (without Ca^{++} Mg^{++})	ThermoFisher	20012050
ROCK inhibitor Y27632	Selleckchem	S1049
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D8418
(Optional) 12-mm glass coverslips**	VWR	89167-106

* Can be substituted with our Neuron Coating Solutions (Coating Materials B and C), Catalog Number: NCS.

** Glass coverslips from different brands might have different effects on the growth of neural cells. We recommend using glass coverslips from Chemglass for the best results.

Workflow



*From Day 7, users may maintain differentiated neurons in the maintenance medium best suited for their needs, though we recommend Quick-Neuron™ GABAergic - Maintenance Medium, Catalog Number: GA-MM.

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.

0.002% Poly-L-Ornithine solution (ornithine)*

1. Take 700 µl 0.01% Poly-L-Ornithine solution and mix it with 2.8 ml PBS.
2. Store the resulting 0.002% Poly-L-Ornithine solution, hereafter referred to as ornithine, for up to 2 weeks at 4°C.

*Ornithine can be substituted with Coating Material B from our Neuron Coating Solutions, Catalog Number: NCS.

1 mg/ml laminin stock solution (laminin)*

1. Thaw Laminin Mouse Protein, Natural and chill PBS at 4°C or on ice.
2. Mix the Laminin Mouse Protein, Natural and PBS to make the 1 mg/ml stock solution, hereafter referred to as laminin.
    - Laminin concentration varies by lot, so use the number specified on the vial or CoA when making your calculations.
3. Make aliquots of a convenient volume (e.g., 35 µl) and store at -20°C.

*Laminin can be substituted with Coating Material C from our Neuron Coating Solutions, Catalog Number: NCS.

Medium N

1. Prepare Medium N using the reagents listed in the table below.
    - Thaw Component N on ice for 20-30 minutes.
    - Thaw all other reagents at room temperature for 20-30 minutes.
    - Store Medium N for up to 2 weeks at 4°C. The leftover reagents can be discarded or saved for other uses.

Medium N Reagents	Volume
DMEM/F12	16 ml
Neurobasal Medium	16 ml
200 mM Glutamax (100x)	167 µl
Penicillin-Streptomycin (10000 units/ml; 100x)	333 µl
Component N	1 ml

Medium N(G1P)

1. Prepare Medium N(G1P) using the reagents listed in the table below.
    - Thaw both vials of Component G1 on ice for 20-30 minutes.
    - Warm/thaw all other reagents at room temperature for 20-30 minutes.
    - **IMPORTANT!** Prepare Medium N(G1P) the day of thawing and plating the cells.

Medium N(G1P) Reagents	Volume
Medium N	11 ml
Component G1	22 µl
Component P	5.5 µl

Medium iN(G1P)

1. Prepare Medium iN(G1P) using the reagents listed in the table below.
    - Warm/thaw Medium N(G1P) and iROCK at room temperature for 20-30 minutes.
    - **IMPORTANT!** Prepare Medium iN(G1P) the day of thawing and plating the cells.

Medium iN(G1P) Reagents	Volume
Medium N(G1P)	6 ml
iROCK	6 µl

Day 0

5-6 hr

Plate/Coverslip Preparation

IMPORTANT! Cells can be plated on glass coverslips or in wells of a 24-well plate depending on the desired application. Wells are better suited for functional assays, whereas coverslips work best for immunostaining and imaging. If using glass coverslips, follow all instructions starting at Step 1. If not, start at Step 1 and skip Steps 2 and 3. Prepare one coverslip or well for each well being passaged.

1. Warm Medium iN(G1P) and thaw ornithine* at room temperature for 20-30 minutes.
2. (Coverslip only) Soak 12-mm glass coverslips and the tips of forceps in 100% ethanol for 3 minutes.
3. (Coverslip only) One by one, air dry each coverslip for 1 minute or until completely dry and put it into a well of the 24-well plate using the sterilized forceps.
4. Vortex ornithine briefly and centrifuge it at a maximum speed for 1 minute.
5. Add 300 µl ornithine to the surface of each glass coverslip or well.
    - **IMPORTANT!** If using coverslips, ensure they are fully submerged under solutions from Step 5 onwards.
6. Incubate the plate at 37°C, 5% CO_2 for at least 2 hours (or at 4°C overnight one day before plating).
7. Thaw laminin* and chill 3.5 mL PBS on ice for 20-30 minutes.
8. Add 35 µl laminin to the chilled PBS. Mix well.
    - All PBS washes should be done dropwise and with room temperature PBS. Chilled PBS is only for the coating step.
9. Aspirate the supernatant from each coverslip/well and add 500 µl PBS.
10. Repeat Step 9.
11. Aspirate PBS from each coverslip/well and add 300 µl diluted laminin.
12. Incubate the plate at 37°C, 5% CO_2 for at least 2 hours or until cells are ready for plating.
13. After the laminin incubation, pipet out most of the supernatant, but not all, from each coverslip or well using a P1000 pipettor.
14. Rinse with 500 µl PBS.
15. Pipet out most of the PBS, but not all, from each well using a P1000 pipettor.
16. Repeat Steps 14-15
17. Add 300 µl Medium iN(G1P) to each well or coverslip using a P1000 pipettor.
18. Incubate the plate at 37°C, 5% CO_2 until cells are ready for plating.

*Ornithine and laminin can be substituted with Coating Materials B and C, respectively, from our Neuron Coating Solutions, Catalog Number: NCS.

Thawing Cells

1. Warm Medium N at room temperature for 20-30 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 min).
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 Medium N to a new 15 ml conical tube.
6. Set a P1000 pipettor to 1 ml but take approximately 500 µl Medium N 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 Medium iN(G1P) 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 10 wells and include a 10% buffer for cell
2. number and volume (a total of 1.1 x 10^6 cells to plate 1 x 10^5 cells in each of the 10 wells). Adjust the volume to 1.1 ml with Medium iN(G1P). If the volume of the cell suspension needed to get 1.1 x 10^6 cells exceeds 1.1 ml,
3. centrifuge the required volume of cell suspension at 200xg for 4 minutes, remove the supernatant, and resuspend the pellet into 1.1 ml Medium iN(G1P).
4. Add 100 µl cell suspension to each of the 10 wells in the 24-well plate using a P200 pipettor.
5. Incubate the cultures at 37°C, 5% CO_2 overnight.

Day 1

 <1 hr

Maintenance

1. Warm Medium N(G1P) at room temperature for 30 min.
2. Pipet out the old medium from each well of the 24-well plate using a P1000 pipettor and add 500 µl PBS to each well along its wall using a P1000 pipettor.
    - **IMPORTANT!** To avoid lifting cells, leave a small volume of the old medium (i.e., just enough to cover the surface of the well). PBS should be applied along the wall of the well very slowly.
3. Pipet out the PBS and add 400 µl Medium N(G1P) to each well along its wall using a P1000 pipettor.
4. Incubate the culture at 37°C, 5% CO_2 overnight.

Day 2

 ~1 hr

Maintenance

1. Prepare Medium N(G2P) using the reagents listed in the table below.
    - Thaw Component G2 on ice for 20-30 minutes.
    - Warm/thaw all other reagents at room temperature for 20-30 minutes.

Medium N(G2P) Reagents	Volume
Medium N	13.5 ml
Component G2	13.5 µl
Component P	6.8 µl

2. Warm Medium N(G2P) at room temperature for 30 min.
3. Pipet out the old medium from each well of the 24-well plate using a P1000 pipettor and add 800 µl Medium N(G2P) to each well along its wall using a P1000 pipettor.
    - **IMPORTANT!** To avoid lifting cells, leave a small volume of the old medium (i.e., just enough to cover the surface of the well). PBS should be applied along the wall of the well very slowly.
4. Incubate the cultures at 37°C, 5% CO_2 for 2 days.

Day 4

 <1 hr

Maintenance

1. Warm Medium N(G2P) at room temperature for 30 min.
2. Pipet out half of the old medium from each well of the 24-well plate using a P1000 pipettor.
3. Slowly add 400 µl Medium N(G2P) to each well using a P1000 pipettor.
4. Incubate the cultures at 37°C, 5% CO_2 for 3 days.

Day 7

Assay or Continuous Maturation

Differentiated neurons can be observed on Day 1-2. For more mature neurons, we recommend culturing cells until Day 7. From Day 7, users may maintain differentiated neurons in the maintenance medium best suited for their needs, though we recommend Quick-Neuron™ GABAergic - Maintenance Medium, Catalog Number: GA-MM. Differentiation into GABAergic neurons can be confirmed with anti-TUBB3 (tubulin beta 3 class III, a global marker for neurons) and anti-GAD67 (glutamic acid decarboxylase, a GABAergic neuron marker) antibodies.