SynFire® Human iPSC-derived induced neurons

Product Information

Description

NeuCyte provides pure ready-to-use **human induced pluripotent stem cells (iPSC)-induced Glutamatergic or GABAergic neurons** expressing typical pan-neuronal markers, e.g. Beta-III Tubulin (TuJ1) and Map2. Excitatory and inhibitory neuronal subtype identities have been confirmed by traditional patch clamping. These human iPSC-derived neurons are especially important because of lack of a natural source of neurons for use in research. These neurons are an essential tool for *in vitro* gain-of-function and loss-of-function genetic studies, as well as for drug development and preclinical studies[1,2,3]. These human neurons can also be used for preclinical safety assessment and chemical neurotoxicity evaluations.

NeuCyte's neurons are suitable for electrophysiological and biochemical assays, for example, to evaluate the effect of compounds on neuronal network activity using multi-electrode arrays (MEA). Different neuronal subtypes can be mixed with human glia for making a defined human mixed neural subtype culture for experimental purposes.

These iPSC-induced neurons are derived from normal human PBMC (peripheral blood mononuclear cells) in a feeder free culture system (passage# 5-10), are cryopreserved and delivered frozen. Once thawed and cultured following our protocols, the cells mature and rapidly exhibit complex neuronal morphologies. Mixed neuronal cultures have synchronous network burst on MEAs within 3 to 4 weeks.

Shipping

Dry ice

Storage and Stability

Store in liquid nitrogen (Astroglia) and -80°C freezer (iNs) immediately upon receipt. This product is stable for at least 6 months from the date of receiving when stored as directed.

Quality Control

Functional validation of maturation and neural network formation potential of iPSC-derived induced neurons has been done on MEAs. All cells have been tested for chromosomal integrity and absence of pathogens (*i.e.* mycoplasma, etc). Full characterization included in the Certificate of Analysis.

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear the appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap, and plastic fragments of the vial.

Restricted Use

This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

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Materials needed but not provided

50% polyethyleneimine (PEI) stock solution (Sigma)
Laminin (Invitrogen, Cat# 23017015)
Axion 48-well MEA Plate
DMEM or DMEM-F12 (Thermo Fisher Cat# 12430062 or 11320082)
Common tissue culture consumables

Material Provided (Please check your package based on the catalog number(s) used for your order.)

Catalog #	Product name	Part #	Component	Product details
1010-1.5	**SynFire Co-Culture Kit (Basic)**	1001-1.5	Glutamatergic induced neurons	>1.5 x 10^6 cells/ vial
		1002-1.5	GABAergic induced neurons	>1.5 x 10^6 cells/ vial
		1003-1.5	Astroglia	>1.5 x 10^6 cells/ vial
		2001-10	Seeding Basal Media	Enough for 10ml
		2001S-10	Seeding Supplement	Enough for 10ml
		2002-20	Short-Term Basal Media	Enough for 20ml
		2002S-20	Short-Term Supplement	Enough for 20ml
		2003-60	Long-Term Basal Media	Enough for 60ml
		2003S-60	Long-Term Supplements	Enough for 60ml
1010-7.5	**SynFire Co-Culture Kit (MEA)**	1001-7.5	Glutamatergic induced neurons	>7.5 x 106 cells/ vial
		1002-3.5	GABAergic induced neurons	>3.5 x 10^6 cells/ vial
		1003-3.5	Astroglia	>3.5 x 10^6 cells/ vial
		2001-20	Seeding Basal Media	Enough for 20ml
		2001S-20	Seeding Supplement	Enough for 20ml
		2002-40	Short-Term Basal Media	Enough for 40ml
		2002S-40	Short-Term Supplement	Enough for 40ml
		2003-120	Long-Term Basal Media	Enough for 120ml
		2003S-120	Long-Term Supplements	Enough for 120ml
1001-10	**SynFire Glutamatergic Induced Neuron Kit (Small)**	1001-1.5	Glutamatergic induced neurons	>1.5 x 10^6 cells/ vial
		1003-1.5	Astroglia	>1.5 x 10^6 cells/ vial
		2001-10	Seeding Basal Media	Enough for 10ml
		2001S-10	Seeding Supplement	Enough for 10ml
		2002-20	Short-Term Basal Media	Enough for 20ml
		2002S-20	Short-Term Supplement	Enough for 20ml
		2003-60	Long-Term Basal Media	Enough for 60ml
		2003S-60	Long-Term Supplements	Enough for 60ml
1001-20	**SynFire Glutamatergic Induced Neurons (Small)***	1001-1.5	Glutamatergic induced neurons	>1.5 x 10^6 cells/ vial

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Catalog #	Product name	Part #	Component	Product details
1002-10	**SynFire GABAergic Induced Neuron Kit (Small)**	1002-1.5	GABAergic induced neurons	>1.5 x 10^6 cells/ vial
		1003-1.5	Astroglia	>1.5 x 10^6 cells/ vial
		2001-10	Seeding Basal Media	Enough for 10ml
		2001S-10	Seeding Supplement	Enough for 10ml
		2002-20	Short-Term Basal Media	Enough for 20ml
		2002S-20	Short-Term Supplement	Enough for 20ml
		2003-60	Long-Term Basal Media	Enough for 60ml
		2003S-60	Long-Term Supplements	Enough for 60ml
1002-20	**SynFire GABAergic Induced Neurons (Small)***	1002-1.5	GABAergic induced neurons	>1.5 x 10^6 cells/ vial
1001-50	**SynFire Glutamatergic Induced Neuron Kit (Large)**	1001-7.5	Glutamatergic induced neurons	>7.5 x 10^6 cells/ vial
		1003-3.5	Astroglia	>3.5 x 10^6 cells/ vial
		2001-20	Seeding Basal Media	Enough for 20ml
		2001S-20	Seeding Supplement	Enough for 20ml
		2002-40	Short-Term Basal Media	Enough for 40ml
		2002S-40	Short-Term Supplement	Enough for 40ml
		2003-120	Long-Term Basal Media	Enough for 120ml
		2003S-120	Long-Term Supplements	Enough for 120ml
1001-60	**SynFire Glutamatergic Induced Neurons (Large)***	1001-7.5	Glutamatergic induced neurons	>7.5 x 10^6 cells/ vial
1002-50	**SynFire GABAergic Induced Neuron Kit (Large)**	1002-3.5	GABAergic induced neurons	>3.5 x 10^6 cells/ vial
		1003-3.5	Astroglia	>3.5 x 10^6 cells/ vial
		2001-20	Seeding Basal Media	Enough for 20ml
		2001S-20	Seeding Supplement	Enough for 20ml
		2002-40	Short-Term Basal Media	Enough for 40ml
		2002S-40	Short-Term Supplement	Enough for 40ml
		2003-120	Long-Term Basal Media	Enough for 120ml
		2003S-120	Long-Term Supplements	Enough for 120ml
1002-60	**SynFire GABAergic Induced Neurons (Large)***	1002-3.5	GABAergic induced neurons	>3.5 x 10^6 cells/ vial

Catalog #	Product name	Part #	Component	Product details
2010-10	SynFire Complete Media Kit (Small)	2001-10	Seeding Basal Media	Enough for 10ml
		2001S-10	Seeding Supplement	Enough for 10ml
		2002-20	Short-Term Basal Media	Enough for 20ml
		2002S-20	Short-Term Supplement	Enough for 20ml
		2003-60	Long-Term Basal Media	Enough for 60ml
		2003S-60	Long-Term Supplements	Enough for 60ml
2010-20	SynFire Complete Media Kit (Large)	2001-20	Seeding Basal Media	Enough for 20ml
		2001S-20	Seeding Supplement	Enough for 20ml
		2002-40	Short-Term Basal Media	Enough for 40ml
		2002S-40	Short-Term Supplement	Enough for 40ml
		2003-120	Long-Term Basal Media	Enough for 120ml
		2003S-120	Long-Term Supplements	Enough for 120ml
2003-1	SynFire Media (Long Term Maintenance)	2003-120	Long-Term Basal Media	Enough for 120ml
		2003S-120	Long-Term Supplements	Enough for 120ml

*We strongly recommend that to achieve optimal performance, the user culture SynFire cells using NeuCyte SynFire media and supplements, instead of media or supplements from any other source.

Please note NeuCyte also provides customized differentiation service. Contact us at **inquiries@neucytelabs.com** for more details.

Important: Before use, add the whole volume contained in each Supplement to its corresponding Basal Media bottle as follows:

1. **For the Seeding Media, add whole content of Seeding Supplement into the Seeding Basal Media bottle (Red labels).**
2. **For the Short-Term Media, add whole content of Short-Term Supplement into the Short-Term Basal Media bottle (Yellow labels). Protect the bottle from light.**
3. **For the Long-Term Media, add whole content of both Long-Term Supplements A and B into the Long-Term Basal Media bottle (Orange labels). Protect the bottle from light.**

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Protocol

Coating of MEA Plates (Axion 48-well MEA plate)

1. Prepare borate buffer (1L): dissolve 3.1 g of boric acid and 4.75 g of sodium tetraborate in distilled water. Adjust pH to 8.4.
2. Prepare 0.1% polyethyleneimine (PEI) solution: dilute 50% PEI stock solution 1:500 in borate buffer. Sterilize 0.1% PEI solution using a 0.22 µm filter.
3. Add 70 µl of prepared 0.1% PEI solution to each well of a 48-well MEA plate. Add approximately 4-5 ml of sterile water to the surrounding areas outside of the wells to reduce media evaporation. Incubate overnight at 37°C.
4. Aspirate PEI solution and thoroughly rinse each well 4 x with 400µl of sterile water.
5. Dilute laminin stock (1mg/ml) to 20µg/ml in PBS.
6. Add 70 µl of prepared laminin solution to each well of the MEA plate and incubate for at least 1 hour at 37°C.
7. Aspirate laminin and directly plate cells.

Thawing of iN and Astroglial Cells

1. To thaw the cells, put the vial in a 37°C water bath with gentle agitation for ~2 minute. Keep the cap out of water to minimize the risk of contamination.
2. Transfer the cells into a 15ml conical tube with pre-warmed (37°C) DMEM-F12 Media. Repeat steps 1-2 for all cell types.
3. Check cell number and viability.
4. The recommended starting number of cells per well of an Axion 48-well plate is 270K (140K Glutamatergic, 60K GABAergic, and 70K Astroglia). Alternatively, the end-user can adjust the ratio of the different cell types to fit their experimental needs.
5. According to the recommended cell density, pool the adequate volumes (adjusted for 50 wells) of each cell type into a new 15ml conical tube, and centrifuge at 250 x g for 5 minutes at room temperature.
6. Aspirate the supernatant and resuspend the cells in 2.5 ml of **Seeding Media**. This volume corresponds to 50 µl/well (adjusted for 50 wells) for subsequent seeding on PEI/laminin-coated MEA plates.

Plating iN and Astroglial Cells into MEA Plates

1. Add 50 µl of pooled cell mix to each well of a previously coated 48-well MEA plate.
2. Incubate seeded MEA plate in a cell culture incubator at 37°C, 5% CO2, and 95% humidity.
3. Next day, add 250 µl of **Short-Term Media** to each well.

Maintaining Human Neural Co-cultures

1. Perform half-medium changes with **Short-Term Media** every other day.
2. At day 7 after seeding, switch medium gradually to **Long-Term Media** by aspirating 150 µl of old medium and adding 150 µl of new medium.
3. Perform half-medium changes with **Long-Term Media** once every 3 days.

Data Acquisition

Spontaneous synchronized neuronal network activity can be measured starting at 3-4 weeks after seeding. Functional neural co-cultures can be maintained for assessment of neuronal activity for up to

8 weeks. Data acquisition for compound screening usually includes measurements of neuronal activity prior to compound application (baseline) and measurements after compound application (dose). Recordings of neuronal activity using the Axion BioSystems MEA platform are performed on the Maestro unit controlled by the AxIS Software according to the manufacturer's protocols.

References

1) ZHANG, Y. et al. Rapid single-step induction of functional neurons from human pluripotent stem cells. **Neuron,** v. 78, n. 5, p. 785-98, Jun 2013.

2) PAK, C. et al. Human Neuropsychiatric Disease Modeling using Conditional Deletion Reveals Synaptic Transmission Defects Caused by Heterozygous Mutations in NRXN1. **Cell Stem Cell,** v. 17, n. 3, p. 316-28, Sep 2015.

3) YI, F. et al. Autism-associated SHANK3 haploinsufficiency causes Ih channelopathy in human neurons. **Science,** v. 352, n. 6286, p. aaf2669, May 2016.