NEXEL

Neurosight®-S User Guide for the Axion Maestro

Contents

Product Information

Unpacking & Handling

- Upon receiving the shipment of Neurosight®-S, check whether all temperature-sensitive components are correctly frozen. If this is not the case, please contact our support team.
- Immediately transfer each of the components to the appropriate storage conditions.
- Please check the catalog number, lot number and expiry date. The basal media expiration date is the shortest (1 year) so experiments should be planned accordingly.
- The Neurosight®-S should be handled by technically qualified individuals complying with good laboratory practices, applicable laboratory regulations and the MSDS. Following the User Guide herein is recommended for best results.
- The Neurosight®-S is intended for research use only, not intended for any type of use in animals or humans.

Components & Description

COMPONENTS	CAT#	STORAGE ON ARRIVAL
Neurosight®-S Neurons		Liquid Nitrogen
Cryopreserved, frozen vial		
>2 million cells	N-001	
>4 million cells	N-002	
Neurosight®-S EP Media		-20°C
Basal medium		
50 ml	NM-003	
100 ml	NM-004	
Neurosight®-S Plating Media		-20°C
20ml	NM-010	
Neurosight®-S Media Supplement (500X)		-20°C
0.13 ml	NS-013	
0.25 ml	NS-014	
Neurosight®-S EP Supplement (50X)		-20°C
1 ml	NS-003	
2 ml	NS-004	
Neurosight®-S Axion MEA User Guide[1]		
Certificate of Analysis (CoA)		
MSDS[2]		

[1] *Also available online at www.nexel.co.kr*

[2] *Enclosed with shipping documents.*

Should any of the above components be missing from your shipment, please contact us at NEXEL Co. Ltd. or the distributor in your country upon which our support team will provide the necessary assistance.

Neurosight®-S Neurons

Cell Type	Human Induced Pluripotent Stem Cell (hiPSC) derived neurons
Cell Line of Origin	hiPSC cell line reprogrammed from commercially available normal donor fibroblast cell line
Quality Control	Please refer to the CoA for lot-specific information. Virus clearance & STR analysis data is available upon request.

Neurosight®-S Media & Media Supplement

- The Neurosight®-S Media & Media Supplement need to be combined to make the Neurosight®-S Maintenance Media, after which it should be used within 1 month. DO NOT FREEZE the Neurosight®-S Maintenance Media, aliquot into smaller quantities for best results.
- The Neurosight®-S Maintenance Media is animal component and serum-free. For additional information on the composition, please contact our technical support team.

- The Neurosight®-S Maintenance Media is antibiotic and antifungal free as they are not necessary if proper conditions are kept. NEXEL does not recommend the use of such agents for accurate results but they should be used if aseptic cell culture conditions are not possible.

Safety Precaution & User Notice

Biosafety Level: 1

For research use only, not intended for any type of use in animal or humans. Appropriate safety procedures should always be used with this material. Please refer to the MSDS for detailed instructions.

User Notice & Restrictions:

- User may use the Product (Neurosight®-S) for internal research including but not limited to screening potential drug compounds for efficacy and safety, and for the provision of such services to third parties. No other right is granted to User whether expressly, by implication, by estoppel or otherwise. In particular, the purchase of the Product does not include nor carry any right or license to use, develop or otherwise exploit the Product commercially, and no rights are conveyed to User to use the Product for any other purpose.
- User agrees to use the Product in compliance with all applicable statutes and regulations, but not to use the Product for any administration or application to humans. Moreover, User agrees not to use the Product in human subjects for human clinical use for therapeutic, diagnostic or prophylactic purposes, or in animals for veterinary use for therapeutic, diagnostic or prophylactic purposes, including but not limited to clinical applications, cell therapy, transplantation, and/or regenerative medicine without an appropriate license.
- In the case that User transfers Product to a third party, User shall convey the User Restrictions set forth herein to such third party.

1. Introduction

NEXEL Co., Ltd. strives to provide high quality human neurons derived from induced pluripotent (iPS) cells using optimized proprietary protocols. The Neurosight®-S is a highly pure and electrophysiologically active population of cells, ensuring researchers get a reliable product. Thus, NEXEL hopes to help the advance of science in tissue-specific research, toxicity screening and drug discovery.

This User Guide will help you seed the Neurosight®-S at the appropriate densities to create mature neuronal networks comprising functional neurons appropriate for a variety of applications related to the electrophysiological behavior optimized to the Axion Maestro platform. In particular, the following protocol will allow users to reliably detect and assess seizurogenic properties of test samples. However, please keep in mind that the best individual results will be obtained by close observation, care and optimization from the user. Example results obtained with the Neurosight®-S can be found below as a reference.

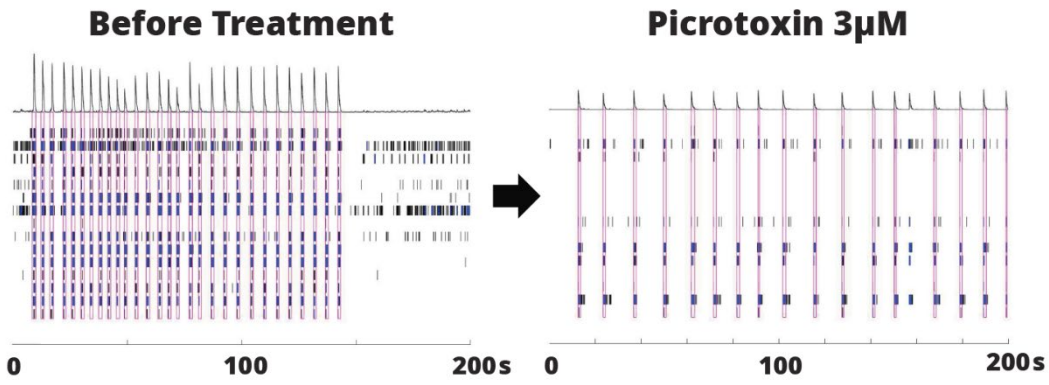

Figure 1. Raster plot of the Neurosight®-S measured using the Axion Maestro before and after treatment of Picrotoxin.

2. Preparing for Cell Culture

Required Equipment and Consumables (Not Provided)

ITEM	CAT#	VENDOR
Axion Maestro (Original, Edge, Pro)	Variable	Axion Biosystems
Axion MEA plate	Variable	Axion Biosystems
Poly-L-ornithine Solution, 50 ml[1,2]	P4957-50ML	Sigma
Laminin Solution, Variable Concentration & Volume[1]	354232	Corning
Typical Cell Culture Equipment Liquid Nitrogen Storage Tank 37°C Water Bath Tabletop Centrifuge Biological Safety Cabinet with UV Lamp Hemocytometer or Automated Cell Counter Phase Contrast Microscope Pipettes Cell Culture Incubator		
Typical Cell Culture Consumables Centrifuge Tubes Cell Culture Plates Pipette Tips Trypan Blue Phosphate Buffered Saline (PBS)		

[1] *It is possible to use materials purchased from other vendors, these are our suggestions for products which are both available world-wide and verified at NEXEL.*

[2] *Other replacements such as Poly-L-lysine or PEI which are used for neuronal cultures can replace Poly-L-ornithine.*

Preparing Neurosight®-S Media

I. Thaw the Neurosight®-S EP and Plating Media by placing at 4°C 24 hours prior to use.

II. In a biosafety cabinet, thaw (at RT) the Neurosight®-S Supplement (500X) and EP supplement (50X) and add it to the thawed media to make Neurosight®-S EP Maintenance Media and Complete Plating Media. Store at 4°C and away from light for up to 2 months. DO NOT FREEZE Neurosight®-S EP Maintenance Media.

> *To avoid oxidation of the media due to air contact and repeated warming/opening, it is recommended to aliquot the media into quantities enough for 2~3 media changes.*

MEDIA TYPE	COMPONENTS
Neurosight®-S EP Maintenance Media	**Neurosight®-S EP Media** **Neurosight®-S Media Supplement** **Neurosight®-S EP Supplement**
Neurosight®-S Plating Maintenance Media	**Neurosight®-S EP Media** **Neurosight®-S Media Supplement**

Preparing MEA Plates

1. Calculate the amount of coating media to be prepared keeping in mind that 50 µl is required for each well. E.g. To coat a whole 48-well plate, prepare a total of 2.5 ml (excess of 100 µl to account for pipetting error).

2. Coat the Axion MEA plates using Poly-L-ornithine solution for > 1 hour at 37°C making sure that the center of each well is covered

   PLO can be recycled and used several times (at least three separate coatings),.

3. Wash the wells twice with PBS (2x the coating volume).

   It is critical to not let it dry before the first wash. After the second wash, while performing step IV, either aspirate immediately or once the Laminin solution is ready.

4. After the second wash, let dry in the incubator at least 3 hours (preferably overnight).

3. Neurosight®-S Thawing and Plating

Thawing

The Neurosight®-S can be thawed using typical cell culture thawing protocols. Here, we present NEXEL's optimized protocol and recommend our users to follow the instructions to maximize results. We strongly recommend thawing 1 vial at a time to minimize cell exposure to liquid DMSO.

I. Calculate the amount of Neurosight®-S Complete Plating Media required. For each vial, 10 ml + the volume required for the first day of culture of Complete Plating Media is required for thawing. The amount required can be calculated by the number of wells; recommendations for different cell culture plates can be found below.

CELL CULTURE PLATE	12-well (3.8 cm^2)	24-well (1.9 cm^2)	48-well (1.0 cm^2)	96-well (0.33 cm^2)
PLATING VOLUME	1 ml	500 µl	300 µl	200 µl

II. Warm the Neurosight®-S Complete plating Media at Room Temperature (RT, 25°C) for at least 30 mins. For each vial to thaw, aliquot 8 ml of Maintenance Media in a 15 ml centrifuge tube.

III. Retrieve the Neurosight®-S vial(s) from the liquid nitrogen storage tank.

IV. Submerge the vial(s) 2/3 in a 37°C water bath so that the mouth of the vial does not come in contact with the water. Constantly check how much has thawed and once ~20% remain (~3 mins), spray the vial(s) with 70% Et-OH, wipe and place it in your biosafety cabinet. Ideally, the vial(s) should have completely thawed exactly when you start step V.

V. Open the vial(s) and transfer the contents (~1 ml) using a 1 ml pipette to the aliquoted 8 ml of Complete Plating Media dropwise while gently swirling the tube.

Dropwise pipetting while gently swirling the tube minimizes osmotic shock and maximizes mixing, which ensures high viability. Drops will remain on the surface for ~1 second and then drop towards the bottom of the tube (visible due to the DMSO content). For dropwise pipetting, simply pipette slowly into the air ~1 cm above the media surface. It should take approximately 1 min per 1 ml.

VI. Use 1 ml of Complete Plating Media to gently rinse the emptied vial and transfer dropwise to the centrifuge tube containing the cells from step V while gently swirling the tube.

VII. Centrifuge the suspended cells at 180 x g for 3 minutes at room temperature.

VIII. Carefully discard the supernatant.

IX. Resuspend the cells gently using 1 ml of Complete Plating Medium and check the cell concentration using a hematocytometer or cell counter and Trypan Blue. Immediately move on to the Plating section.

> *Avoid rigorous pipetting of the cells to maximize viability. Single cell resuspension of the Neurosight®-S during thawing should easily be achieved by gently pipetting 3~4 times.*

Plating onto the MEA plate

I. Thaw the cells according to the Neurosight®-S User Guide using the Neurosight®-S Plating Media.

II. Calculate the amount of total cells required, keeping in mind that 50,000 cells are required for each well. For example, to plate a whole 48-well plate, calculate the volume that corresponds to 2,500,000 cells (excess of 100,000 cells; 50 wells total).

III. Transfer the corresponding volume to a 1.5 ml tube.

IV. Centrifuge the suspended cells at 180 x g for 3 minutes at room temperature.

V. During the centrifugation, remove the D-PBS which had been added around the wells. This will allow for easier handling of the plate when seeding the cells.

VI. Resuspend the cells using Neurosight®-S Plating Media to match the plating density (5 µl per 50,000 cells). E.g. For 50 wells, 250 µl is the correct volume.

VII. Add Laminin to a final concentration of 10 µg/ml in the cell resuspension

VIII. Hold the plate at an angle which allows to see the electrode grid in each well. Pipette the correct amount of cells (5 µl/50,000 cells) to the center of the wells in a manner that forms a drop over the measurement electrodes. This step determines the seeding placement of the cells – covering all the measurement electrodes is best. It is preferable to avoid covering the T-shaped reference electrodes.

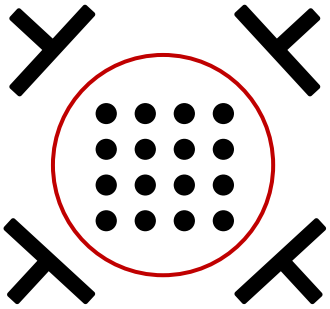

Figure 2. Droplet Placement Diagram
Independently of the plate type, all Axion plates are shaped in a similar manner with smaller round measuring electrodes in the center and reference electrodes on the outside. A 5 µl droplet will cover the measurement area appropriately as shown in red.

IX. Handle the plate gently and add 6~8 ml of D-PBS around the wells to increase the humidity. Should the droplet dry out, cells will not be able to attach properly.

X. Incubate at 37°C for exactly 30 minutes.

XI. Add 300 µl of pre-warmed Neurosight®-S Complete Plating Media complemented with 1 µg/ml Laminin to each well.

4. Neurosight®-S Maintenance

Starting the day after plating the cells, the media needs to be changed every two to three days. Ideally, the media should be changed at 48 or 72-hour intervals. When performing electrophysiological assays on the cells, we recommend changing the media 1 hour prior to the experiment to ensure there are enough nutrients for the cells.

I. Warm the correct volume of Neurosight®-S EP Maintenance Media volume (half of the volume for plating) at Room Temperature for at least 30 mins.

II. Immediately before media change, add Laminin to media to a final concentration of 1 µg/ml.

III. Perform a half-media change with the newly warmed media in a biosafety cabinet. Pipette softly onto the cell culture plate walls to avoid any damage to the cell culture.

Performing half-media changes is crucial for neuronal cultures. Firstly, even the shortest contact with air can damage pure neuronal cultures and cell death will be visible within a few days. Secondly, it prevents removal of cytokines secreted by the cells themselves resulting in higher quality cell cultures. An example of half-media change is: for 1 ml of media in the well, remove ~ 400 µl and add 500 µl.

Neuronal cultures are very sensitive to pipetting. Both upon removal and addition of media, users should take extra care to not result in detachment of the culture.

IV. Place the plate back in the incubator.

V. Repeat I to IV every 2~3 days.

We recommend performing any planned assays on the Axion Masetro with the Neurosight®-S from Day 21 onwards. Long term culture over 21 days can lead to aggregation at high densities but users should not be alarmed as aggregation on its own is not always detrimental to experiment results. If cell attachment is unstable after long term culture, please check the state of the laminin being used and whether half-media changes are being performed gently.