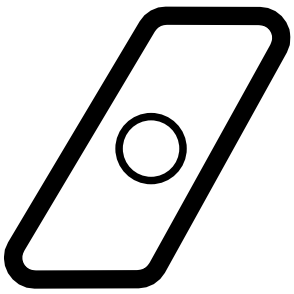
NEXEL

Neurosight®-S
User Guide



Contents

1. 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.
- 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 (date of expiration on label) 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		
Cryopreserved, frozen vial		Liquid Nitrogen
> 2 million cells	N-001	
> 4 million cells	N-002	
Neurosight®-S Maintenance Media		
Maintenance medium		-20 °C
50 ml	NM-001	
100 ml	NM-002	
Neurosight®-S Maintenance Supplement		
0.5 ml	NS-001	-20 °C
1.0 ml	NS-002	
0.15 ml	NS-013	
0.25 ml	NS-014	
Neurosight®-S EP Media		
MEA* maintenance medium		-20 °C
50 ml	NM-003	
100 ml	NM-004	
Neurosight®-S EP Media Supplement (50X)		
1.0 ml	NS-003	-20 °C
2.0 ml	NS-004	
Neurosight®-S Plating Media		
MEA* Plating medium		-20 °C
20 ml	NM-010	
Neurosight®-S 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 Supplements

- The Neurosight®-S Media & Supplements 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, but aliquot into smaller quantities for best results.
- The above also applies for Neurosight®-S EP Media and Supplements, and Plating Media and Supplements.
- The Neurosight®-S Maintenance Media are serum-free. For additional information on the composition, please contact our technical support team.
- The Neurosight®-S Maintenance Media are 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.

2. Introduction

NEXEL Co., Ltd. strives to provide high quality human neurons derived from induced pluripotent stem (iPS) cells using optimized proprietary protocols. The Neurosight®-S is a highly pure and electrophysiologically active population of cells, suitable for all types of experiments in the field of neuron research. As such, they are the perfect choice to test the advance of science in tissue-specific research, toxicity screening, efficacy testing, and drug discovery.

This User Guide will help you seed the Neurosight®-S at the appropriate densities to create mature neuronal networks comprised of functional neurons appropriate for a variety of applications related to the electrophysiological behavior such as calcium fluxes or MEA assays and neurite outgrowth or degeneration. However, please keep in mind that the best individual results will be obtained by close observation, care, and optimization from the user. Example morphology of the Neurosight®-S can be found below as a reference (Figure 1).

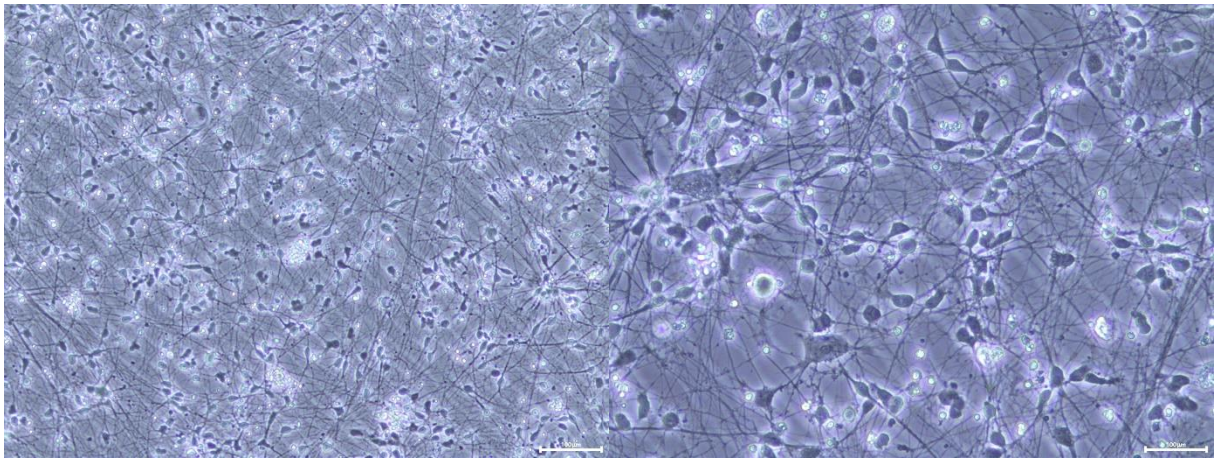


Figure 1. Morphology of Neurosight®-S at day 14 of culture. Image on the left was taken at 100X magnification, whereas the image on the right was taken at 200X magnification.

3. Preparing for Cell Culture

Required Equipment and Consumables (Not Provided)

ITEM	CAT#	VENDOR
Coating Material		
Poly-D-Lysine	A3890401	Gibco
Laminin	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)		

Preparing Neurosight®-S Media

1. Thaw the Neurosight®-S Media and Supplements by placing them at 4°C 24 hours prior to use.
2. In a biosafety cabinet, thaw and add the Neurosight®-S Maintenance Supplement to the thawed medium to make corresponding Media. Store at 4°C for up to 1 month after addition of the supplement. DO NOT FREEZE Neurosight®-S 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 Maintenance Media	**Neurosight®-S Maintenance media** **Neurosight®-S Maintenance Supplement**
Neurosight®-S EP Media	**Neurosight®-S EP media** **Neurosight®-S EP Supplement**
Neurosight®-S Plating Media	**Neurosight®-S Plating media**

Preparing Cell Culture Surfaces

1. Calculate the amount of coating media required using the following table as a reference.

CELL CULTURE PLATE	6-well (9.6 cm^2)	12-well (3.8 cm^2)	24-well (1.9 cm^2)	48-well (1.0 cm^2)	96-well (0.33 cm^2)	MEA 48 well Plate
COATING VOLUME	1 ml	500 µl	300 µl	100 µl	50 µl	50 µl

2. Pipette appropriate amount of Poly-D-Lysine solution to each well.

   ※ Do not swirl the MEA plate

3. Incubate the plate at 37°C CO_2 incubator overnight.
4. Next day, remove the Poly-D-Lysine solution in each well.
5. Rinse two times with sterile PBS. As for the MEA 48-well plate, let it completely dry in the biosafety cabinet (over 2 hours).
6. Prepare the laminin coating solution to working concentrations immediately before use as described in the following table. Both coating solutions can be kept at 4°C for a short period of time but this is not recommended.

COATING TYPE	STOCK CONCENTRATION	WORKING CONCENTRATION
Laminin	**Varies by bottle. Check label for concentration.**	**5 µg/ml**

7. Pipette the correct amount of coating solution to each well you intend to use. For MEA plates, we recommend pipetting 5 µl of laminin solution on top of the electrodes.
8. Gently swirl the plate and check whether all wells are completely covered.

   ※ For MEA plates, DO NOT swirl the plate.

9. Incubate at 37°C for at least an hour.

   ※ For MEA plates, incubating the laminin coating for 1 hour is critical. Please refer to the "**Neurosight®-S Application Protocol for the Axion Maestro MEA**" for more detail.

4. 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.

1. Calculate the amount of Neurosight®-S Maintenance Media required. For each vial, 10 ml of Maintenance Media is required for resuspending the vial. The amount of Maintenance Media required can be calculated by the number of wells; recommendations for different cell culture plates can be found below.

CELL CULTURE PLATE	6-well (9.6 cm^2)	12-well (3.8 cm^2)	24-well (1.9 cm^2)	48-well (1.0 cm^2)	96-well (0.33 cm^2)	**MEA 48-well plate**
PLATING VOLUME	2 ml	1 ml	500 µl	300 µl	200 µl	300 µl

2. Warm the Maintenance 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.
3. Retrieve the Neurosight®-S vial(s) from the liquid nitrogen storage tank.
4. Submerge the vial(s) 2/3 in a 37 °C water bath so that the mouth of the vial does not 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 5.
5. Open the vial(s) and transfer the contents (~1 ml) using a 1 ml pipette to the aliquoted 8 ml of Maintenance 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 minute per 1 ml.
6. Use 1 ml of Maintenance Media to gently rinse the emptied vial and transfer dropwise to the centrifuge tube containing the cells from step 5 while gently swirling the tube.
7. Centrifuge the suspended cells at 180 x g for 3 minutes at room temperature.

8. Carefully discard the supernatant. Be careful not to disturb the cell pellet at bottom of the tube to avoid any cell loss.
9. Resuspend the cells gently using 1 ml of Maintenance Media 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

NEXEL recommends seeding the Neurosight®-S at a density of ~100,000 cells/cm^2 for most standard applications. Application specific protocols are available upon request. The best results are obtained by the User's own optimization, for which NEXEL will try to provide as much assistance as possible.

1. Calculate the volume of Maintenance Media and cells required to match the correct density for the culture platform of choice. Below is a table with cell numbers.

CELL CULTURE PLATE	6-well (9.6 cm^2)	12-well (3.8 cm^2)	24-well (1.9 cm^2)	48-well (1.0 cm^2)	96-well (0.33 cm^2)	**MEA 48-well Plate**
PLATING VOLUME	2 ml	1 ml	500 µl	300 µl	200 µl	300 µl
CELL NUMBER	960,000	380,000	190,000	100,000	33,000	100,000

Well area (*cm^2*) can vary between different vendors, please check with your providers for exact calculations.

2. Combine the volumes as calculated above. Add laminin to a final concentration of 1 µg/ml.
3. Remove the coating solution in the cell culture plates. Avoid drying out the coated wells as much as possible.
4. Gently mix by pipetting and evenly distribute the appropriate volumes of cells with Maintenance Media.
5. Move the cell culture plate to the incubator, shake the plate in perpendicular directions to evenly distribute the cells for attachment.
6. The day after, perform the first media change with Maintenance Media (Neurosight®-S Maintenance Step 1).

***For MEA plates, follow the plating procedure below.**

1. Calculate the number of cells required for seeding.
2. Centrifuge and dissolve in media, to a final concentration of 1.0 x 10^5 cells per 5 µl
3. Carefully remove the laminin coating solution and immediately add 5 µl of the cell suspension to the coated area.
4. Incubate for at least 30 min at RT in the biosafety cabinet.
5. After incubation, carefully add 300 µl of Maintenance Media.

5. Neurosight®-S Maintenance

Starting 24 hours after plating the cells, the media needs to be changed every two to three days. Ideally, the media should be changed at 48-hour intervals. When performing electrophysiological assays on the cells, we recommend changing the media on the morning of the experiment to ensure there are enough nutrients for the cells and to deliver the desired drug concentration.

1. Warm the correct volume of Neurosight®-S Maintenance Media volume (half of the volume for plating) at room temperature for at least 30 mins.
2. Immediately before media change, add Laminin media to a final concentration of 1 µg/ml.
3. 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. First, it prevents even the shortest contact with air which can damage neuronal cultures. If the cell is exposed to air, cell death will be visible within a few days. Second, it prevents removal of cytokines secreted by the cells, which results 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.

   - While performing half-media changes, it is critical to keep the plate flat on a surface. Tilting the plate during half-media changes can cause the cells to shift position and potentially induce aggregation towards the tilted direction.

   - For MEA plates, replace a full volume of Plating Media with EP Media 24 hours after plating. To remove the spent media, aspirate the media using a pipette without tilting the plate. Leave a small amount of media so that the cells do not come in contact with air. Then gently add appropriate amount of pre-warmed EP Media. After the initial full-media change, perform half-media changes on 48-hour interval.

4. Place the plate back in the incubator.
5. Repeat 1 to 3 every 2~3 days.

We recommend performing any planned assays with the Neurosight®-S from Day 14 onwards. Long-term culture over 28 days can lead to aggregation or cell detachment, we recommend careful and constant observation of the cell culture. Aggregation on its own is not always detrimental to experiment results, particularly in electrophysiological assays. We do not recommend subculture/passaging of the neuronal cell culture as mature neurons are very fragile and neurite destruction may lead to cell death.