Neurosight-S®

user guide

WE MAKE HUMAN CELLS

Contents

User Notice & Restrictions:

1. User may use the Product 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.
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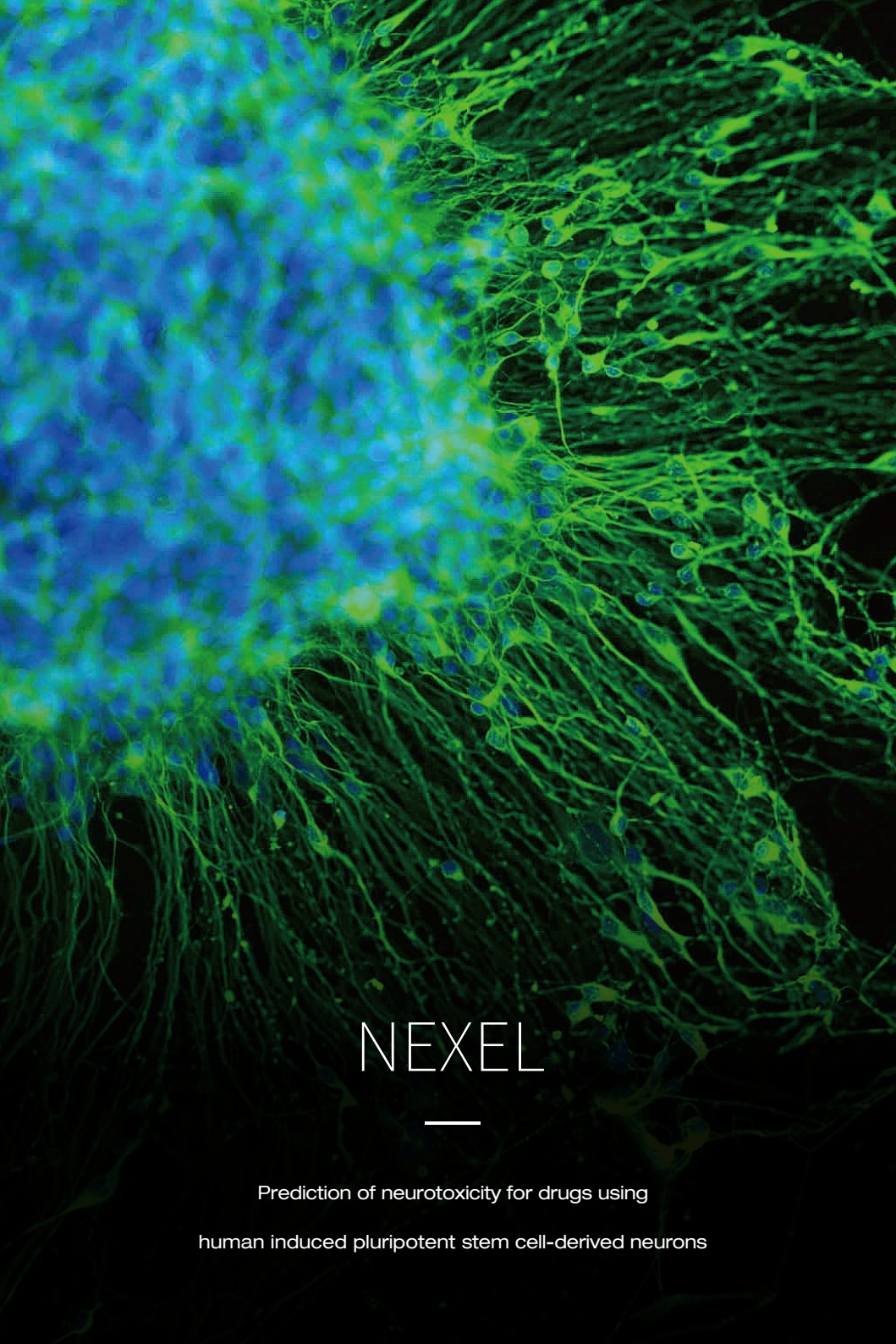
2. User agrees to use the Product in compliance with all applicable statutes and regulations, but not to use the Product for any administration or application to humans.
   Moreover, User agrees not to use the Product in human subjects for human clinical use for therapeutic, diagnostic or prophylactic 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.

3. In the case that User transfers Product to a third party, User shall convey the User Restrictions set forth herein to such third party.



NEXEL

Prediction of neurotoxicity for drugs using

human induced pluripotent stem cell-derived neurons

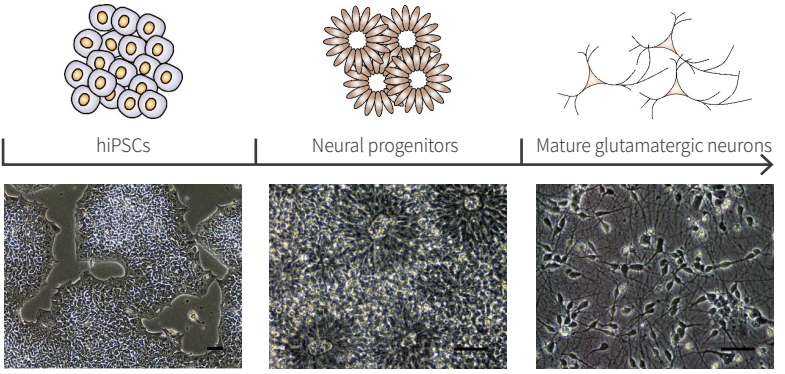
1. Introduction

Neurosight-S® is a highly pure population of glutamatergic neurons differentiated from human induced pluripotent stem cells (hiPSCs).

2. Characteristics of Neurosight-S®

2-1. Morphology

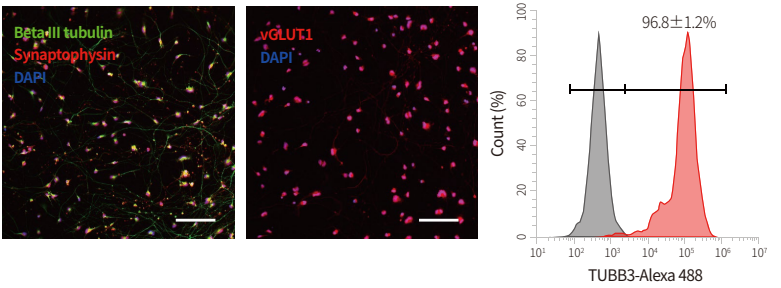
The Neurosight-S® was induced using NEXEL's proprietary protocol *in vitro.*



scale bar 50μm

Summary of Neurosight-S® induction process and representative images of cell morphology at each step.

2-2. Cytochemical Analysis



Neurosight-S® expresses glutamatergic neuron markers (TUBB3, SYP, vGLUT1) and is a highly pure population of mature neurons (TUBB3 ≥ 95%).

3. List of Components (provided by NEXEL)

Neurosight-S® (N-001)

Item	Cat. #	Storage	Note
Neurosight-S®	N-001	≤-150°C	hiPSC-derived neurons, ≥2 X 10^6cells
Neurosight-S® Media	NM-001	4°C	50 ml
Neurosight-S® Supplement (100X)	NS-001	≤-20°C	500 µl

Neurosight-S® (N-002)

Item	Cat. #	Storage	Note
Neurosight-S®	N-002	≤-150°C	hiPSC-derived neurons, ≥4 X 10^6cells
Neurosight-S® Media	NM-002	4°C	100 ml
Neurosight-S® Supplement (100X)	NS-002	≤-20°C	1 ml

4. Additional Materials Required (Not provided by NEXEL)

Item	Vendor	Cat. #
Poly-L-ornithine solution	Sigma	P4957
Laminin	Sigma	L2020
PBS	Multiple Vendors	-

These specific items are highly recommended to be used for most optimal result

5. Storage

- Neurosight-S® should be stored at ≤−150°C (Liquid Nitrogen tank).
- Neurosight-S® Media should be stored at 4°C and used before the expiry date indicated on the label
- Neurosight-S® Supplement should be stored at -20°C.

6. Preparing Cell Culture

6-1. Media

Type of Media	Components
Thawing Media	Neurosight-S® Media
Maintaining Media	Neurosight-S® Media + Neurosight-S® Supplement (100X)

Note: For the Maintaining Media, use 1X of the Neurosight-S® Supplement .

6-2. Plate (coating matrix)

1. Choose the desired cell culture plates and coat with the poly-L-ornithine (PLO) solution to each well by adding recommended volume

Cell culture plate	6 well	12 well	24 well	48 well	96 well
Recommended amount / well	1000 µl	500 µl	300 µl	100 µl	50 µl

2. Distribute liquid evenly by gently swirling the plate.
3. Incubate the plate at 37°C incubator for at least 1 hour.
4. After incubation, completely aspirate the PLO solution from each well, and then rinse 2 times with sterile PBS.
5. Prepare laminin solution to the final concentration of 5ug/ml by diluting in sterile PBS.
6. Add diluted laminin solution into the PLO-coated well using recommended volume
7. Incubate the plate at 37°C incubator for at least 1 hour.
8. Before plating the cells, completely aspirate the laminin solution from each well, and immediately add pre-warmed Maintaining Media.

Note: Avoid repeated warming of Neurosight Media to ensure the maximal performance

Cell culture plate	6 well	12 well	24 well	48 well	96 well
Recommended volume for Maintaining	2 ml	1 ml	600 ul	300 ul	100 ul

7. Thawing Procedure

1. Warm the Thawing Media required for thawing (total 5ml per 1vial) in 37°C water bath.
2. Remove the Neurosight-S® cryovial from the liquid nitrogen storage tank.
3. Put the cryovial in 37°C water bath until the cells begin to thaw (until small ice clump is left)
   Note: The vial should not be completely submerged in water bath
4. Remove the cryovial from the water bath, spray with 70% EtOH.
5. After wiping with cleaning paper, place the vial in a safety hood.
6. Discard the cap, gently add 500ul of fresh Thawing Media into the thawed vial, and mix together by pipetting several times.
7. Gently transfer the thawed cells into 15 ml conical tube containing 4.5ml of Thawing Media.
8. Pipet slowly to mix the cell suspension.
9. Rinse the empty vial to recover any residual cells.
10. Centrifuge the suspended cells at 300g(1200rpm) for 3 minutes at room temperature.
11. Carefully discard the supernatant.
12. Add 1ml of Maintaining Media into the tube, then, gently pipet for cell resuspension.

Note: Avoid repeated pipetting of thawed Neurosight-S® to ensure the maximum cell recovery.

8. Plating Neurosight-S®

1. Confirm the number of viable cells using trypan blue exclusion method with hemocytometer or automated cell counter.
2. Calculate the final volume of cell suspension to obtain the desired cell plating density

Note: Recommended count for Neurosight-S® plating is $5x10^4$~$1x10^5$ cells/cm^2.

Cell culture plate	6 well	12 well	24 well	48 well	96 well
Growth area	9.6 cm^2	3.8 cm^2	1.9 cm^2	0.75 cm^2	0.33 cm^2

3. Gently distribute the cell suspension into the cell culture plate.
4. After 1day, confirm the cell attachment and then fully replace the Maintaining Media.
5. Incubate the plated cells in cell culture incubator at 37°C, 5% CO_2.

9. Maintaining Neurosight-S®

1. Warm the Maintaining Media in 37°C water bath before use.

Note: Avoid repeated warming of Neurosight Media to ensure the maximal performance

2. For subsequent Maintaining, half-media change (50~70%) is recommended.

Cell culture plate	6 well	12 well	24 well	48 well	96 well
Recommended volume for Maintaining	2 ml	1 ml	600 ul	300 ul	100 ul

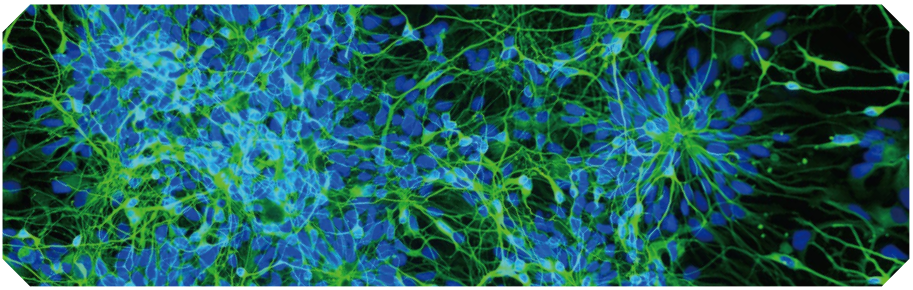
3. Culture the cells for 7~14 days with Maintaining Media (Replace the media every 2~3 days), and use the Neurosight-S® for your research purpose.

Note: For extended culture more than 14days, addition of laminin (1ug/ml) in Neurosight Media is suggested to prevent accidental neuron detachment.

Note: We recommend to add the Maintaining Media slowly to ensure maximum viability and attachment

Licensed Patents

AJ No.	Country	Application No.	Patent No.
AJ001	Japan	2007-550210	5098028
AJ001	Japan	2008-131577	4183742
AJ001	Japan	2009-056747	4411362
AJ001	Japan	2009-056750	4411363
AJ001	Japan	2009-056748	5248371
AJ001	Japan	2009-056749	5467223
AJ001	Japan	2011-088113	5603282
AJ001	Japan	2013-167725	5943324
AJ001	China	200680048227.7	200680048227.7
AJ001	China	201010126185.2	201010126185.2
AJ001	China	201310015158.1	201310015158.1
AJ001	China	201410006027.1	Patent Pending
AJ001	Hong Kong	09102406.5	1125131
AJ001	Hong Kong	09103541.9	1125967
AJ001	India	3564/CHENP/2008	Patent Pending
AJ001	Korea	10-2008-7017015	10-1420740-0000
AJ001	Singapore	200804231-9	0143419



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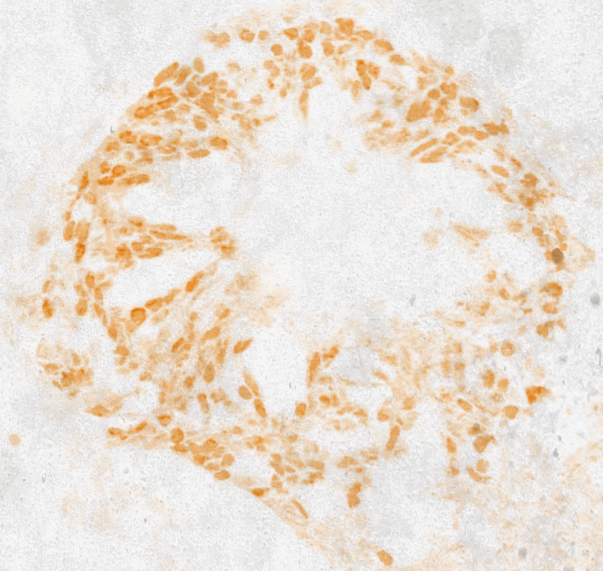

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