



## Poietics™ Neural Progenitor Cell System

### NHNP – Instructions for Use

---

#### Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. Remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact Customer Service.
3. BulletKit™ Medium instructions: store basal medium at 2°-8°C and SingleQuots™ Kit at ≤20°C in a freezer that is not self-defrosting. Once thawed SingleQuots™ Kit should be stored at 2°-8°C and added to basal medium within 72 hours. After SingleQuots™ Kit is added to basal medium, use within 1 month. Do not re-freeze. Using medium or reagents other than what is recommended will void the cell warranty. Please contact Scientific Support if you need help selecting media and/or reagents.

#### NHNP Culture Overview

Poietics™ NHNP cells are provided as cryopreserved neurospheres from primary cultures. For best performance, we recommend thawing NHNP cells into Neural Progenitor Maintenance Media (NPMM™ BulletKit™ Medium; Catalog No. CC-3209). After a minimum of 24 hours in the NPMM™ Maintenance Medium, differentiation may be induced by transferring neurospheres to cultureware coated with mouse laminin (not included, available from Trevigen or R&D Systems) in Neural Progenitor Differentiation Media (NPDM BulletKit™ Medium; Catalog No. CC-3229).

Differentiation is enhanced by supplementation of NPDM with various differentiation-promoting agents. NPDM should be supplemented with brain-derived neurotrophic factor (BDNF, not included, available from R&D Systems) at a final concentration of 25 ng/ml, or another neurotrophic factor of your choice.

Subculture of neurosphere suspensions by trypsinization is not recommended, as increased attachment and spontaneous differentiation may occur. Repeated subculture of neurospheres may lead to a reduced percentage of neuronal cells upon differentiation.

#### Preparation of Media

1. Decontaminate external surfaces of all vials and the medium bottle with ethanol or isopropanol.
2. To formulate Neural Progenitor Maintenance Media (NPMM™ Maintenance Medium) transfer the contents of the NHNP Growth SingleQuots™ Kit (Catalog No. CC-4241 containing human recombinant Basic Fibroblast Growth Factor [rhFGF-B] and human recombinant Epidermal Growth Factor [rhEGF]) and the contents of the NHNP Supplement SingleQuots™ Kit (Catalog No. CC-4242 containing Neural Survival Factor-1 [NSF-1] and Gentamicin/Amphotericin-B [GA-1000]), to NPBM™ Basal Medium with a pipette, and rinse each vial with medium.
3. To formulate Neural Progenitor Differentiation Media (NPDM) transfer the contents of the NHNP Supplement SingleQuots™ Kit (Catalog No. CC-4242 containing Neural Survival Factor-1 [NSF-1] and Gentamicin/Amphotericin-B

[GA-1000]), to NPBM™ Medium with a pipette, and rinse each vial with medium.

4. When preparing these BulletKit™ Media, it may not be possible to recover the entire volume listed for each vial. Small losses (up to 10%) should not affect the cell growth characteristics of the supplemented medium.
5. NPDM must be further supplemented with differentiation-promoting agents. We recommend brain-derived neurotrophic factor (BDNF, R&D Systems) at 25 ng/ml.
6. Transfer the label provided with each kit to the basal medium bottle(s) being supplemented (avoid covering the basal medium lot # and expiration date). Use it to record the date and amount of each supplement added.

## Cell Thawing / Initiation of Suspension Culture

1. Prepare one T-150 flask or two T-75 flasks for each NHNP cryovial. Add the appropriate amount of medium (40 ml of NPMM™ Medium/ T-150 flask or 20 ml of NPMM™ Medium per T-75 flask) and allow the vessels to equilibrate in a humidified 37°C incubator with 5% CO<sub>2</sub> for at least 30 minutes.
2. Prior to thawing the cells, place the cryovial in a sterile field and briefly twist the cap a one-fourth turn to relieve pressure, and then re-tighten. Quickly thaw the cryovial in a 37°C water bath, being careful not to submerge the entire vial. Wipe cryovial with ethanol or isopropanol before opening. Watch your cryovial closely; when the last sliver of ice melts, remove it. Do not submerge it completely. Thawing the cells for longer than 2 minutes results in less than optimal results.
3. Using a 2 ml pipette, re-suspend the cells in the cryovial and dispense them into the equilibrated flask(s). Do not wash cells before plating, as centrifugation is more damaging than residual DMSO in the culture. Gently rock the flask(s) to evenly distribute the cells and return to the incubator.

## Plating/Differentiation

Dissociation of neurospheres prior to plating is not necessary to induce differentiation. Spheres will attach to laminin-coated culture plates in BDNF supplemented NPDM and cells will migrate outward and differentiate into a monolayer of neuronal and

glial cells. After cells are maintained in flask(s) for 24 hours, do the following:

1. Calculate the number of plates required for seeding based on the desired cell culture format (e.g., 24-well plate), the number of cells quoted on Certificate of Analysis, and a recommended seeding density of 30,000 cells/cm<sup>2</sup>.
2. Coat cell culture plates with mouse laminin (Trevigen or R&D Systems) at 5 µg/ cm<sup>2</sup>. Consult the documentation provided by the laminin manufacturer for detailed procedures and alternative protocols. Alternatively, pre-coated cultureware is commercially available. Coated plates should be rinsed with differentiation medium immediately prior to the addition of the cells.
3. Warm 1 ml of differentiation medium (i.e., NPDM supplemented with BDNF) to room temperature per every 60,000 cells.
4. Centrifuge neurosphere suspension at low speed (50-60 x g) for 5 minutes. Carefully remove supernatant and gently but thoroughly re-suspend cells in the pre-warmed differentiation medium.
5. Transfer 0.5 ml/cm<sup>2</sup> of cell suspension to the cell culture plates. Larger neurospheres will rapidly sediment, so it is recommended that cells be dispensed one well at a time with continuous gentle pipetting of the stock suspension.
6. Place the plates in a humidified 37°C incubator with 5% CO<sub>2</sub>. Spheres should attach within 24 hours and cells should begin outward migration from the spheres after a few days.

## Maintenance After Plating

1. Change differentiation medium every 72 hours. Avoid dislodging cells.
2. For best results, run assays when cells have reached 70% confluence, generally within 9-14 days. If culture becomes too dense, neuritic processes may retract.

## Quality Control

All cells are performance assayed and test negative for HIV-1, mycoplasma, Hepatitis-B and C, bacteria, yeast and fungi. Cell viability, cell number and morphology are measured after recovery from cryopreservation. Poietics™ Media are formulated for optimal support of specific types of normal

human cells. Certificates of Analysis (COA) for each cell strain are shipped with each order. COAs for all other products are available upon request.

## Ordering Information

### Neural Progenitor Cells (Single Donor):

Cat. No.	Product	Description
PT-2599	NHNP, cryopreserved	$\geq 1.2 \times 10^6$ cells

### Neural Progenitor Media (Sold Separately):

Cat. No.	Product	Description
CC-3209	NPMM™ BulletKit™ Medium	200 ml NPBM™ Basal Medium plus CC-4241 and CC-4242 SingleQuots™ Kits to formulate NPMM™ Medium (maintenance medium)
CC-3229	NPDM BulletKit™ Medium	200 ml NPBM™ Basal Medium plus CC-4242 SingleQuots™ Kit to formulate NPDM (differentiation medium)
CC-3210	NPBM™ Basal Medium	Neural progenitor cell basal medium (200 ml)
CC-4241	NHNP Growth SingleQuots™ Kit	Formulates 200 ml of NPBM™ Basal Medium to NPMM™ Maintenance Medium when used with CC-4242 SingleQuots™ Kit
CC-4242	NHNP Supplement SingleQuots™ Kit	Formulates 200 ml of NPBM™ Basal Medium to differentiation medium

## Product Warranty

Cultures have a finite lifespan *in vitro*.

Lonza guarantees the performance of its cells in the following manner only if Poietics™ Media and Reagents are used exclusively, and the recommended protocols are followed. The performance of cells is not guaranteed if any modifications are made to the complete cell system.

1. Poietics™ NHNP are assured to be viable and functional when thawed and maintained properly.
2. NHNP are cryopreserved in primary passage as neurospheres. Routine characterization of NHNP includes positive immunofluorescence for GFAP and Beta-Tubulin III following differentiation. Lonza guarantees NHNP will express the markers described when plated out

of cryopreservation onto laminin-coated plates in the presence of BDNF.

When placing an order or to contact Scientific Support, please refer to the product numbers and descriptions listed above. For a complete listing of all Clonetics™ Products, refer to the Lonza website or the current Lonza catalog. To obtain a catalog, additional information or Scientific Support, you may contact Lonza by web, e-mail, telephone, fax or mail (See page 1 for details).

**THESE PRODUCTS ARE FOR RESEARCH USE ONLY.** Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

**WARNING: CLONETICS™ AND POIETICS™ PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS.** Each donor is tested and found non-reactive by an FDA-approved method for the presence of HIV-1, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human-sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, [Biosafety in Microbiological and Biomedical Laboratories, 5<sup>th</sup> ed.](#) If you require further information, please contact your site safety officer or Scientific Support.

All trademarks herein are marks of Lonza Group or its subsidiaries.