Neural Stem Cell Expansion Kit - Neurosphere

Catalog Number SC003

Reagents for the expansion of neural stem cells.

This package insert must be read in its entirety before using this product.

**FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
THE SAFETY AND EFFICACY OF THIS PRODUCT IN DIAGNOSTIC
OR OTHER CLINICAL USES HAS NOT BEEN ESTABLISHED.**

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R&D Systems, Inc.
614 McKinley Place NE
Minneapolis, MN 55413
United States of America

TELEPHONE: (800) 343-7475
(612) 379-2956
FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

R&D Systems Europe, Ltd.
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB
United Kingdom

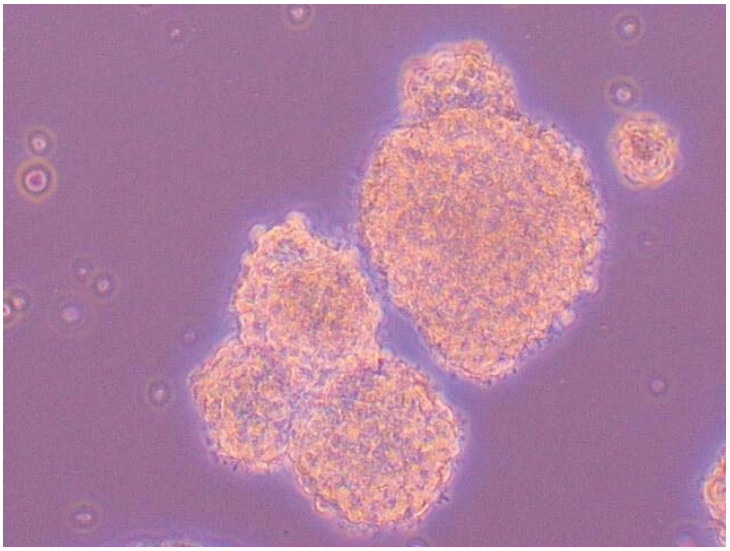
TELEPHONE: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

R&D Systems China Co. Ltd.
24A1 Hua Min Empire Plaza
726 West Yan An Road
Shanghai PRC 200050

TELEPHONE: +86 (21) 52380373
FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Neural stem cells (NSCs), with the potential to self-renew and to differentiate into neurons and glial cells, have been isolated from different regions of the developing and adult brain (1 - 6). NSCs can be grown *in vitro*, in the presence of mitogen, as either monolayer cultures on an adhesive substrate or as free-floating spherical aggregates termed 'neurospheres'. These *in vitro* propagated NSCs can proliferate in culture while retaining the potency to differentiate into neurons, oligodendrocytes and glia. *In vitro* NSC expansion makes it possible to grow sufficient numbers of NSCs for researchers to study basic developmental biology, drug discovery and preclinical neural transplantation.



Expanded rat NSC in the neurosphere system on day 4 after passage 5.

DESIGN OF THE KIT

The Neural Stem Cell Expansion Kit - Neurosphere is a system designed for *in vitro* neural stem cell expansion as neurospheres in a serum-free environment. The kit contains a specially formulated N-2 Plus Media Supplement, which has been optimized for neural stem cell expansion (7). Two growth factors, human epidermal growth factor (EGF) and human fibroblast growth factor basic (FGF basic), are included to promote the *in vitro* proliferation of neural precursors. The quantity of each component provided in the kit is estimated to be sufficient to make 500 mL of growth medium for cell expansion. This kit is suitable for the neurosphere culture model and is applicable for human, rat and mouse cells. Cells propagated using this kit are Nestin-positive (8) and retain their potency to differentiate into neurons, oligodendrocytes and glia, as identified after growth factor withdrawal by staining with the antibodies Tuj1, O4 and anti-GFAP, respectively.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- The safety and efficacy of this product in diagnostic or other clinical uses have not been established.
- The kit should not be used beyond the expiration date on the kit label.
- The quality of the neural stem cells and any variation in the procedure can cause variation in the efficiency of cell expansion.

PRECAUTIONS

The acute and chronic effects of overexposure to reagents of this kit are unknown. Safe laboratory procedures should be followed and protective clothing should be worn when handling kit reagents.

The N-2 Plus Media Supplement contains human transferrin. This transferrin was tested at the donor level using an FDA licensed method and found to be non-reactive for anti-HIV-1/2 and Hepatitis B surface antigen. As no testing can offer complete assurance of freedom from infectious agents, these reagents should be handled as if capable of transmitting infection.

REAGENTS

N-2 Plus Media Supplement (Part 390155) - 5 mL of a 100X concentrated solution, containing bovine insulin, human transferrin, sodium selenite, putrescine, and progesterone.

Human EGF (Part 390186) - 20 μg of lyophilized recombinant human epidermal growth factor in PBS.

Human FGF basic (Part 390187) - 20 μg of lyophilized recombinant human fibroblast growth factor basic in 20 mM Tris-HCl (pH 7.0) containing 50 μg of bovine serum albumin per 1 μg of cytokine.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at ≤ -20° C in a manual defrost freezer. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="3">Opened Reagents</td><td>N-2 Plus Media Supplement</td><td rowspan="3">Store at 2 - 8° C for up to 1 month or aliquot and store at ≤ -20° C in a manual defrost freezer for up to 6 months.* Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Human EGF</td></tr>
<tr><td>Human FGF basic</td></tr>
</table>

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

Materials

- Neural Stem Cells (prepare according to references: 7, 9 - 11)
- 0.2 μm, 500 mL filter unit (Nalgene, VWR Catalog # 28198-505 or equivalent)
- 6-well plates (Costar Catalog # 3516 or equivalent)
- 15 mL tubes (Corning Catalog # 430052 or equivalent)
- Pasteur pipettes (Fisher Catalog # 13-678-20C or equivalent)
- Pipettes and pipette tips

Reagents

- DMEM/F-12, no HEPES (Invitrogen® Catalog # 12500-062, 11320-033, or equivalent)
- PBS (Invitrogen Catalog # 10010-023 or equivalent)
- Glucose (Sigma Catalog # G6152 or equivalent)
- L-Glutamine (Sigma Catalog # G5763 or equivalent)
- Sodium Bicarbonate, $NaHCO_3$ (Sigma Catalog # S5761 or equivalent)
- Penicillin-Streptomycin, 100X (Invitrogen Catalog # 15140-148 or equivalent)
- BSA, very low endotoxin (Millipore Catalog # 81-068-3 or equivalent)
- Acetic acid (J.T. Baker Catalog # 9508-03 or equivalent)
- Deionized water

Equipment

- 37° C and 5% CO_2 incubator
- Centrifuge
- Hemocytometer
- Microscope

REAGENT AND MATERIAL PREPARATION

N-2 Plus Medium - Mix the following ingredients with deionized water to make 500 mL of medium. Adjust pH to 7.2 ± 0.2. Sterile filter the solution using a 500 mL, 0.2 μm filter unit, and add 5 mL of 100X Penicillin-Streptomycin. Store in the **dark** at 2 - 8° C for up to 2 weeks.

DMEM/F-12*	6 g (495 mL*)
Glucose	775 mg
L-Glutamine	36.5 mg
$NaHCO_3$	845 mg
N-2 Plus Media Supplement (100X)	5 mL

*Liquid DMEM/F-12 (Invitrogen Catalog # 11320-033; no HEPES) can be used as an alternative to the powder form (Invitrogen Catalog # 12500-062).

10 mM Acetic Acid - Add 10 μL of acetic acid (17.4 M concentrate) into 17.4 mL of sterile deionized water.

0.1% BSA in 10 mM Acetic Acid - Dissolve 10 mg of BSA into 10 mL of 10 mM acetic acid. Sterile filter the solution by syringe filter and store at 2° - 8° C for up to 3 months.

0.1% BSA in PBS - Dissolve 10 mg of BSA into 10 mL of PBS. Sterile filter the solution by syringe filter and store at 2° - 8° for up to 3 months.

EGF Stock (1000X) - Add 1 mL of sterile 10 mM acetic acid containing 0.1% BSA to the Human EGF vial. Aliquot and store at 2° - 8° C for up to 1 month or at ≤ -20° C in a manual defrost freezer for up to 6 months. Avoid repeated freeze-thaw cycles.

FGF basic Stock (1000X) - Add 1 mL of sterile PBS containing 0.1% BSA to the Human FGF basic vial. Aliquot and store at 2° - 8° C for up to 1 month or at ≤ -20° C in a manual defrost freezer for up to 6 months. Avoid repeated freeze-thaw cycles.

N-2 Plus/EGF/FGF basic Medium - Dilute EGF Stock and FGF basic Stock 1000-fold in N-2 Plus Medium. Prepare fresh as needed.

PROCEDURE

Use serological pipettes to transfer and remove solutions.

NSC Expansion - Neurosphere

1. Seed approximately 1 x 10^5 NSCs in 5 mL of N-2 Plus/EGF/FGF basic Medium per well in a 6-well plate.
2. Incubate the cells at 37° C and 5% CO_2.
3. Supplement the medium with EGF (final concentration of 1X) and FGF basic (final concentration of 1X) each day. Every fourth day, based on the number of neurospheres, replace the medium according to the steps described below.
   a. ≥ 50 neurospheres - Transfer the medium containing the neurospheres to a 15 mL tube. Centrifuge for 5 minutes at 40 x g. Remove the supernate. Add 1 mL of fresh N-2 Plus/EGF/FGF basic Medium and gently resuspend the pellet, without dissociating the neurospheres. Add the neurosphere suspension to 4 mL of fresh N-2 Plus/EGF/FGF basic Medium in one well of a 6-well plate.

   b. < 50 neurospheres - Transfer the neurospheres, using a Pasteur pipette, directly into 2.5 mL of fresh N-2 Plus/EGF/FGF basic Medium in one well of a 6-well plate. **DO NOT DISCARD THE MEDIUM FROM THE ORIGINAL WELL(S)**, but add it to the neurospheres to bring the final volume to 5 mL, which can be measured using a serological pipette.
4. Pass the cells at 7 - 15 days, or when the neurospheres have a dark clump inside or ruffling on the outside of the neurosphere, according to the procedure described below.

NSC Passage

1. Transfer the medium containing the neurospheres to a 15 mL tube.
2. Centrifuge for 5 minutes at 40 x g. Remove approximately 4.5 mL of the supernate.
3. Partially dissociate the neurospheres in the remaining supernate by pipetting up and down with a 200 µL pipette tip.
4. At the initial passages 1 and 2, add 5 mL of fresh N-2 Plus/EGF/FGF basic Medium to the partially dissociated neurospheres and transfer the final neurosphere suspension into one well of a 6-well plate. Repeat step 3 in the NSC Expansion section (see above).
5. After passage 2, add 10 mL of fresh N-2 Plus/EGF/FGF basic Medium to the partially dissociated neurospheres and split the final neurosphere suspension into 2 wells of a 6-well plate (5 mL of neurosphere suspension/well). Repeat step 3 in the NSC Expansion section (see above).

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NOTES

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