NeuroXVivo™

Rat Cortical Neuron Culture Kit

Catalog Number CDK011

Reagents for culturing primary rat cortical neurons.

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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USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Cortical neuron culture is an important model system for studies of neuronal development and function, neurotoxicity screening, drug discovery, and mechanisms of neurological diseases. Proper development and survival of neurons, *in vivo* as well as *in vitro*, requires specific growth factors, signaling molecules, peptides, and vitamins (1, 2). The NeuroXVivo™ Rat Cortical Neuron Culture Kit provides optimized reagents and growth factors, as well as a validated protocol, to successfully culture rat cortical neurons in a serum-free environment. The kit contains a Neuronal Base Media, a neuronal media supplement, and growth factors to support the robust growth and maintenance of both short- and long-term neuronal cultures. This kit is designed to work with primary rat cortical neurons isolated during either embryonic or postnatal stages. The NeuroXVivo™ Rat Cortical Neuron Culture Kit is a simple and reliable tool for culturing primary rat cortical neurons for both experienced and non-experienced users.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store unopened kit at ≤ -20 °C in a manual defrost freezer. Do not use past kit expiration date.

COMPONENTS	PART #	# VIALS	STORAGE OF OPENED/RECONSTITUTED MATERIAL
Neuronal Base Media	967977	1 vial (500 mL)	Store at 2-8 °C under sterile conditions for up to 30 days or at -20 °C to -70 °C in a manual defrost freezer for up to 3 months.*
N21-MAX Media Supplement (50X)	893414	1 vial (10 mL)	
Recombinant human BDNF	967978	1 vial	
Recombinant human IGF-I	967979	1 vial	
Reconstitution Buffer 1	967552	2 vials (1 mL/vial)	Store at 2-8 °C under sterile conditions for up to 3 months.*

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

OTHER MATERIALS & SUPPLIES REQUIRED

- E17–E18 Timed Pregnant Rat or P1-P2 Rat Pups
- Poly-D-Lysine (R&D Systems®, Catalog # 3439-100-01)
- Poly-L-Lysine –coated µ-slides
- Mouse Laminin-I (R&D Systems®, Catalog # 3400-010-01)
- L-Glutamine
- Antibiotic-Antimycotic (100X)
- Phosphate Buffered Saline (PBS)
- Sterile deionized or distilled water (dH_2O)
- Parafilm
- Fire-polished glass pasteur pipette
- Tissue culture plates
- Inverted microscope
- Dissection microscope
- Dissection tools
- Hemocytometer
- 37 °C, 5% CO_2 incubator
- Laminar flow cell culture hood
- Centrifuge
- Pipettes and pipette tips
- Conical tubes
- 37 °C water bath

ADDITIONAL REAGENTS FOR POSTNATAL NEURON CULTURE

- Papain
- DNAse-I
- Ovomucoid Protease Inhibitor
- Earle's Balanced Salt Solution

REAGENT PREPARATION

Neuronal Base Media - Thaw at 37 °C.

N21-MAX Supplement (50X) - Thaw at 2-8 °C.

Recombinant Human BDNF (1000X) - Add 560 µL of Reconstitution Buffer 1 to the vial of Recombinant Human BDNF to produce Recombinant Human BDNF (1000X).

Recombinant Human IGF-I (1000X) - Add 600 µL of Reconstitution Buffer 1 to the vial of Recombinant Human IGF-I to produce Recombinant Human IGF-I (1000X).

Complete Cortical Neuron Culture Media - Add N21-MAX Supplement (50X) at a final concentration of 1X to the desired amount of Neuronal Base Media (e.g., for every 100 mL of base media, add 2 mL of N21-MAX Supplement (50X)). Add Recombinant Human BDNF (1000X) and Recombinant Human IGF-I (1000X) at a final concentration of 1X to the desired amount of Neuronal Base Media (e.g., for every 100 mL of base media, add 100 µL each of Recombinant Human BDNF (1000X) and Recombinant Human IGF-I (1000X)). Supplement media with 0.5 mM L-Glutamine and Antibiotic-Antimycotic (1X).

Note: *Complete Cortical Neuron Culture Media is stable for up to 1 month at 2-8 °C after adding all the growth supplements.*

PROTOCOL FOR RAT CORTICAL NEURON CULTURE

Note: *Rat cortical neurons can be cultured in plastic tissue culture plates or on glass slides. When culturing cells in plastic tissue culture plates, the entire protocol must be followed. When culturing cells on Poly-L-Lysine–coated µ-slides, Steps A and B can be skipped.*

A. POLY-D-LYSINE (PDL): COATING OF PLASTIC TISSUE CULTURE PLATES

1. Dilute the PDL stock solution with sterile dH_2O to the final concentration of 50 µg/mL.
2. Pipette enough diluted PDL solution into tissue culture plates of choice to cover the surface area (e.g., pipette 50 µL/well for 96-well plate).
3. Rock gently to ensure even coating across the surface of the culture plate.
4. Incubate coated plates in a 37 °C, 5% incubator for approximately 1 hour.
5. Remove the PDL solution by aspiration and wash with sterile dH_2O three times.
6. After the third wash, remove water completely by aspiration.
7. Seal-wrap plates with Parafilm and store at 2-8 °C for up to 2 weeks.

B. PREPARATION OF CORTICAL NEURON CULTURE PLATES

Note: *This step should be started the day before rat dissection*.

1. Prepare Mouse Laminin-I coating solution in PBS at 10 µg/mL.
2. Pipette enough solution into tissue culture plates of choice, which have been previously coated with PDL (See Step A), to cover the surface area (e.g., pipette 50 µL/well for 96-well plate).
3. Incubate coated tissue culture plates at 2-8 °C overnight.
4. On the day of dissection and prior to adding cells to the plate, remove Mouse Laminin-I solution by aspiration and wash with sterile dH_2O twice.

C. NEURONAL DISSECTION

1. Warm an appropriate amount of Neuronal Base Media and Complete Cortical Neuron Culture Media in a 37 °C water bath. The volume warmed depends on the number of plates or slides to be plated. **Note:** *See table 1 on page 5.*

2. Autoclave dissection tools before use to sterilize them.

3. Dissect cortical neurons from E17-18 rat embryos or from P1-2 rat pups. If using P1-2 rat pups, skip step a) and b) below.

    a. Recover the embryos from a pregnant rat by C-section after CO_2 asphyxiation. Place the embryos in a large petri dish containing ice-cold PBS and place on ice.

    b. Remove the embryos from their individual placenta sacs and clean them with ice-cold PBS to remove any remaining blood.

    c. Place cleaned embryos in a large petri dish containing ice-cold PBS and carefully decapitate just at the position where the neck meets the head. For P1-2 pups, quickly decapitate using sharp scissors. Place the heads in another large petri dish with ice-cold PBS. Discard the decapitated bodies in the appropriate bio-waste container.

    d. Position and stabilize the dissociated head using both the angled and straight forceps and carefully cut through the skull with the small surgical scissors going in the caudal to rostral direction. Be careful not to cut too deep so as not to damage the brain tissue.

    e. Carefully peel back the two halves of the separated skull and locate the whole brain.

    f. Carefully remove the whole brain from the head cavity using angled forceps and place on ice in a small petri dish containing PBS. Repeat the same for the remaining heads, if applicable.

    g. To begin dissection of the brain cortex, place an individual brain in another small petri dish containing ice cold PBS and, under the dissecting microscope, separate both hemispheres of the brain by cutting down the median longitudinal fissure with micro-dissection scissors. Cut off and discard any remaining brain stem tissue.

    h. Gently peel off the meninges that cover each brain hemisphere using straight forceps. Open up the brain tissue to reveal the mid-sagittal side of the brain.

    i. Using a micro-dissection scissors, remove and discard the hippocampus (which is c-shaped and a little darker than the surrounding tissue). Discard the dissected hippocampus and place the remaining cortical tissue in a separate petri dish containing ice cold PBS. Place on ice. Repeat for the rest of the brains.

C. NEURONAL DISSOCIATION AND CULTURE

1. After the cortical tissues have been recovered, cut them into smaller pieces (~2 mm^2) and process into single cell suspension as follows:

    a. For embryonic cortical neuron, place cortical tissues in a 15 mL tube and add 5 mL of Neuronal Base Media. Dissociate brains into individual cells by triturating 10-15 times using a fire-polished pasteur pipette. After trituration, the cells should be suspended evenly in solution.

    b. For postnatal cortical neuron, tissues need to be enzymatically digested before trituration in order to yield a healthy cell population. In 5 mL of EBSS make up an enzyme solution containing 20 U/mL Papain and 100 U/mL DNase-I. Warm the solution in a 37 °C, 5% CO_2 incubator for 10 minutes. Place cortical tissues in the pre-warmed enzymatic solution. Incubate the vial in a 37 °C, 5% CO_2 incubator for 20-30 minutes. After digestion, dissociate brains into individual cells by triturating 10-15 times using a fire-polished pasteur pipette. After trituration, the cells should be suspended evenly in solution.

2. Pellet the cells by centrifugation for 5 minutes at 200 x g at room temperature. Discard the supernatant and resuspend the cells in 10 mL of Neuronal Base Media for embryonic cortex or in 5 mL of Ovomucoid Protease Inhibitor (1 µg/mL in EBSS) for postnatal cortex. Pellet the cells by centrifugation for 4-6 minutes at 200 x g room temperature.

3. Wash the cells twice with 10 mL of Neuronal Base Media. Pellet the cells by centrifugation for 5 minutes at 200 x g room temperature.

4. Discard the supernatant and resuspend the cells in pre-warmed Complete Cortical Neuron Culture Media (recommended volume is 10 mL). Count the cells and determine the cell density needed for the culture or assay. Dilute neuron suspension to desired seeding density (See Table1) using pre-warmed Complete Cortical Neuron Culture Media.

Table 1. Recommended seeding densities for cortical neuron culture.

CULTURE DISHES/ CHAMBER SLIDES	MEDIUM VOLUME/ WELL (mL)	TOTAL NUMBER OF CELLS REQUIRED TO SEED EACH WELL		
		Low Density (2.5×10^4 cells/cm^2)	Medium Density (5.0×10^4 cells/cm^2)	High Density (2.5×10^5 cells/cm^2)
6-well plate	2.0	2×10^5	5×10^5	2×10^6
12-well plate	0.8	1×10^5	2×10^5	1×10^6
24-well plate	0.5	5×10^4	1×10^5	5×10^5
48-well plate	0.3	2.5×10^4	5×10^4	2.5×10^5
96-well plate	0.1	5×10^3	2×10^4	5×10^4
4-well µ-slide	0.5	5×10^4	1×10^5	5×10^5
8-well µ-slide	0.3	2×10^4	4×10^4	2×10^5

C. NEURONAL DISSOCIATION AND CULTURE *CONTINUED*

5. Seed neuron suspension onto a Poly-D-Lysine/Laminin-I pre-coated tissue culture plate or a Poly-L-Lysine-coated µ-slide.

6. Culture cortical neurons for the desired amount of time by exchanging media every 3-4 days. Healthy cultures can be maintained for up to 4 weeks.

7. Exchanging media:

    a. Warm an appropriate amount of Complete Cortical Neuron Culture Media in a 37 °C, 5% CO_2 incubator.

    b. Using a pipette, gently remove half the volume of media from cultured cortical neuron plate or vessel (e.g., in a 96-well plate, for each well remove 50 µL from a total volume of 100 µL). Do not remove the whole volume as this causes stress to neurons.

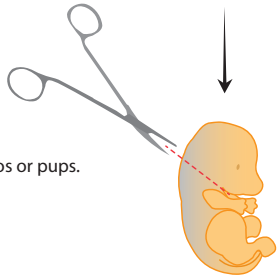
    c. Add an equal amount of fresh pre-warmed Complete Cortical Neuron Culture Media to the cultured cortical neuron plate or slide.

    d. Perform media exchange every 3-4 days.

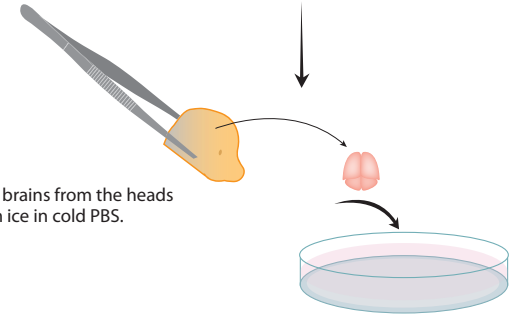
DISSECTION OUTLINE

Isolate E16–18 rat embryos or P1–2 rat pups.

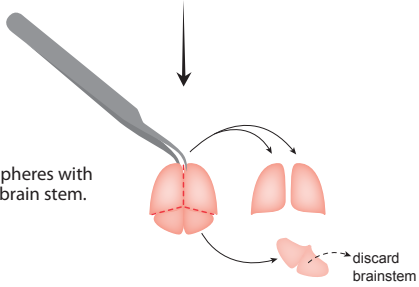
Decapitate rat embryos or pups.



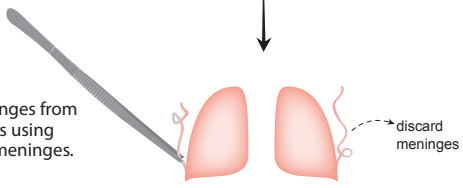
Remove the brains from the heads and place on ice in cold PBS.



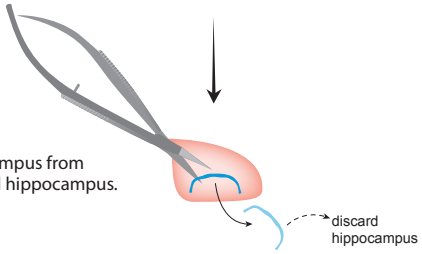
Separate the cortical hemispheres with curved forceps and discard brain stem.



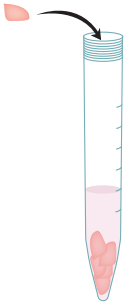
Peel off the meninges from both hemispheres using forceps. Discard meninges.



Cut out C-shaped hippocampus from both hemispheres. Discard hippocampus.

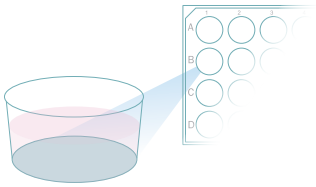


Collect cortical tissues in a conical tube containing Neuronal Base Media.

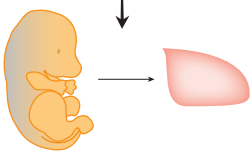


PROTOCOL OUTLINE

Day 1
Prepare tissue culture plates by coating with Poly-D-Lysine and Laminin-I.

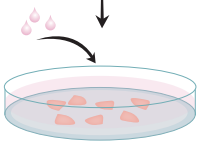


Day 2
Isolate cortical tissue from E16–18 rat embryos or P1–2 pups following the dissection protocol outline.

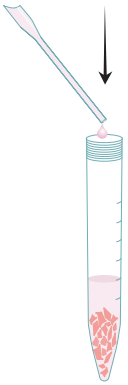


Digest cortical tissue with Papain and DNase-1 for 20–30 minutes.*

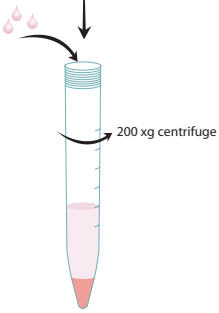
*Only if using brains from postnatal rats.



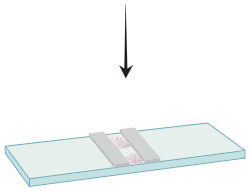
Collect tissues in a conical tube containing Neuronal Base Media and dissociate into a single-cell suspension using a fire-polished pasteur pipette.



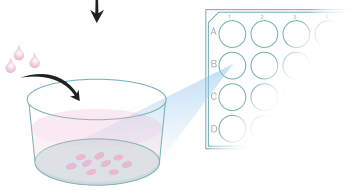
Pellet the cells by centrifugation at 200 x g. Suspend cells in Neuronal Base Media and pellet cells–repeat a total of two times.



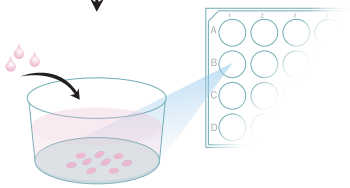
Suspend cell pellet in **Complete Cortical Neuron Culture Media**. Perform a cell count.



Suspend desired amount of cells per milliliter in **Complete Cortical Neuron Culture Media**. **Seed** cells onto Poly-D-Lysine/ Laminin-I coated tissue culture plates or µ-slides.



Culture cortical neurons for desired amount of time. Exchange media every 3–4 days.



DATA EXAMPLES

Note: *Representative results below were obtained from culturing E18 rat cortical neurons in vitro with this kit for 14 days.*

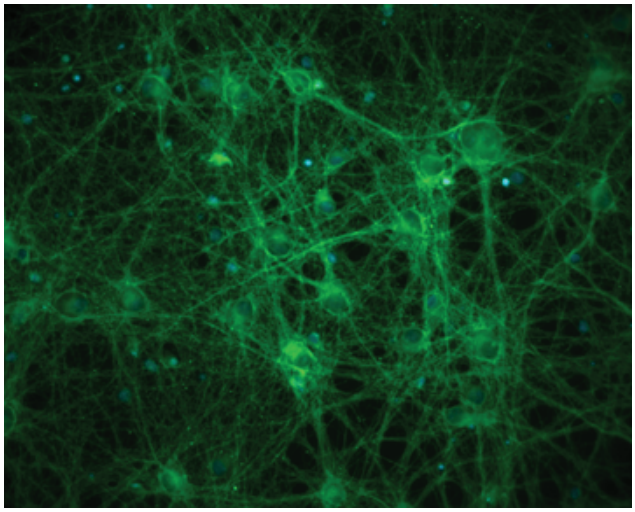


Figure 1: Rat Cortical Neurons Express beta-III Tubulin and Form an Extensive Network of Neuronal Processes. Cortical neurons were stained with the Neuron-specific beta-III Tubulin NorthernLights™ (NL)493-conjugated Antibody (R&D Systems®, Catalog # NL1195G; green) and counterstained with DAPI (blue).

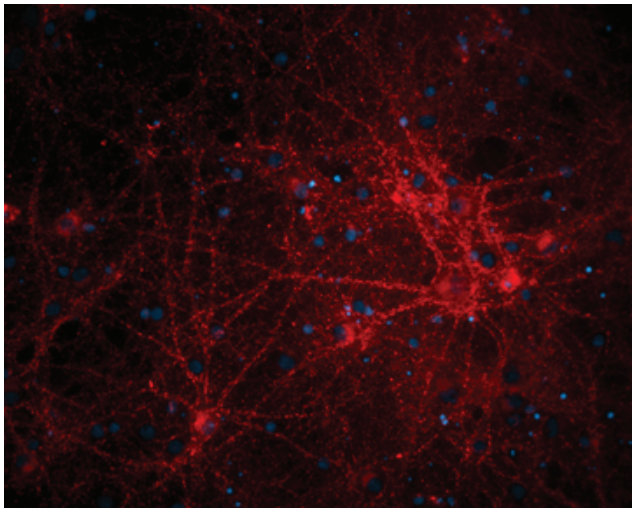


Figure 2: Synapse Formation in Embryonic Rat Cortical Neurons in Culture. Synaptic puncta were visualized in cultured rat cortical neurons using a Mouse Anti-Rat Synaptotagmin-1 Antibody (R&D Systems®, Catalog # MAB4364), followed by the NorthernLights™ (NL)557-conjugated Donkey Anti-Mouse IgG Secondary Antibody (R&D Systems®, Catalog # NL007; red) and counterstained with DAPI (blue).

DATA EXAMPLES *CONTINUED*

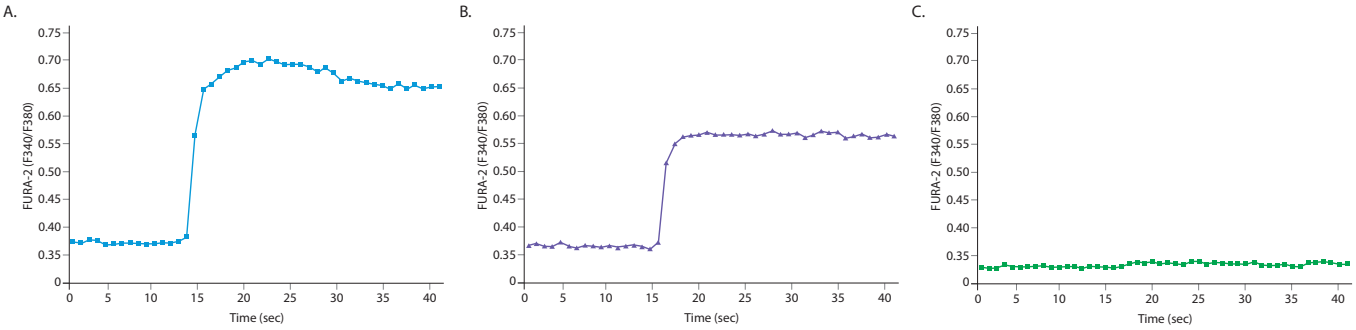


Figure 3: Depolarization Mediated Intracellular Calcium Response in Cultured Rat Cortical Neurons induced with Glutamate and Potassium Chloride. Representative traces of intracellular Ca^{2+} response (F340/F380) in Fura-2 loaded rat cortical neurons in culture upon addition of **(A)** 3 µM Glutamate, **(B)** 50 mM Potassium Chloride, or **(C)** HBSS control.

SUGGESTED REAGENTS FOR IMMUNOCYTOCHEMISTRY

DESCRIPTION	CATALOG NUMBER
Neuron-specific beta-III Tubulin NorthernLights™ (NL)493-conjugated Antibody	NL1195G
Human GFAP NorthernLights™ (NL)557-conjugated Antibody	NL2594R
Rat Synaptotagmin-1 Antibody	MAB4364
NorthernLights™ (NL)557-conjugated Donkey Anti-Mouse IgG Secondary Antibody	NL007
Human CaM Kinase II alpha Antibody	MAB5584

REFERENCES

1. Catapano, L.A., *et.al.* (2001) J. Neurosci. **21**:8863
2. Martin, D.L. (1992) Glia. **5**:81