Primary Rat Cortex and Hippocampus Neurons

Catalog Numbers A10840, A10841, A36511, A36512, A36513

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Primary Rat Cortex and Hippocampus Neurons are isolated from day-18 Fisher 344 rat embryos and cryopreserved in a medium containing DMSO. Primary Rat Neurons are the flexible, ready-to-use and quality alternative to freshly isolated neurons.

Contents and storage

Contents	Cat. No.	Amount	Storage
Primary Rat (Fisher 344) Cortex Neurons, 1 × 10^6 viable cells/vial (RCN IM)	A10840-01	1 mL	Liquid nitrogen, vapor-phase
Primary Rat (Fisher 344) Cortex Neurons, 4 × 10^6 viable cells/vial (RCN IM)	A10840-02		
Primary Rat (Fisher 344) Hippocampus Neurons, 1 × 10^6 viable cells/vial (RHN IM)	A10841-01		
Primary Rat (Sprague Dawley) Cortex Neurons, 1 × 10^6 viable cells/vial (RCN IM SD)	A36511		
Primary Rat (Sprague Dawley) Cortex Neurons, 4 × 10^6 viable cells/vial (RCN 4M SD)	A36512		
Primary Rat (Sprague Dawley) Hippocampus Neurons, 1 × 10^6 viable cells/vial (RHN IM SD)	A36513		

Important guidelines for thawing and storing cells

- Upon receipt, immediately thaw cells or place into vapor-phase liquid nitrogen storage until ready to use. **Do not store the cells at –80°C**.
- Avoid short-term extreme temperature changes. When storing cells in liquid nitrogen after shipping on dry ice, allow the cells to remain in liquid nitrogen for 3-4 days before thawing.

Procedural guidelines

- The following procedures are designed for neuronal cells grown in completeNeurobasal™ Plus Medium. Results may differ with culture systems grown in other complete medium formulations which can result in higher number of non-neuron cells (i.e. astrocytes).
- We recommend using Neurobasal™ Plus Medium for primary rat neuron cultures. Add GlutaMAX™ I Supplement and B-27™ Plus Supplement to Neurobasal™ Plus Medium prior to use to prepare complete Neurobasal™ Plus Medium:
- For primary rat hippocampus neuron cultures: Supplement the complete Neurobasal™ Plus Medium with an additional 25 µM L-Glutamate up to day 4 in culture.
- **IMPORTANT!** Do not vortex cells at any time during this procedure.
- **IMPORTANT!** Do not expose neurons to air at any time.

Culture conditions

Medium: Complete Neurobasal™ Plus Medium

Cell lines: Primary Rat Cortex and Hippocampus Neurons

Culture type: Adherent

Culture vessels: Poly-D-Lysine (4.5 µg/cm^2) coated plates

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 5% CO_2 in air

Prepare complete Neurobasal™ Plus Medium

1. Aseptically add 200 mM GlutaMAX™ I Supplement to a final concentration of 0.5 mM (2.5 mL/L) to the Neurobasal™ Plus Medium before use.

2. Aseptically add B-27™ Plus Supplement (50X) to a final concentration of 2% (v/v) (20 mL/L) to the Neurobasal™ Plus Medium before use.

3. (Optional): **For primary rat hippocampus neuron cultures**: Supplement the complete Neurobasal™ Plus Medium (prepared as described above) with an additional 25 µM L-Glutamate up to day 4 in culture.

Recover and culture primary rat neurons

IMPORTANT! Do not vortex at any time during this procedure.

1. Rinse a 15-mL conical culture tube with pre-warmed (37°C) complete Neurobasal™ Plus Medium and leave it in the cell culture hood prior to thawing the cells.

2. If you are removing a vial from liquid nitrogen storage, twist cap slightly to release pressure and then re-tighten cap.

   Note: Thaw one vial at a time. Transfer the vial immediately from liquid nitrogen storage to a 37°C water bath, minimizing handling time. You may use an ice-bucket containing dry ice to transport the vials from liquid nitrogen to the water bath. Use forceps to transfer the vial.

3. Rapidly thaw (< 2 minutes) the frozen vial by gently swirling it in a 37°C water bath.

   Remove the vial from the water bath when only a tiny ice crystal is left. (The vial should be still cold to touch).

4. Transfer the vial to the cell culture hood and disinfect it with 70% isopropyl alcohol.

   Tap the vial gently on the surface of the hood so that the liquid settles down to the bottom of the vial.

5. Rinse a 1-mL pipette tip with complete Neurobasal™ Plus Medium and very gently transfer the cells to the pre-rinsed 15-mL tube (from step 1).

6. Rinse the vial with 1 mL of complete Neurobasal™ Plus Medium (pre-warmed to 37°C) and add to the cells in the 15-mL tube extremely slowly at the rate of one drop per second.

   Mix the suspension by gentle swirling after each addition.

   Note: Do not add the entire amount of medium to the tube at once. This may lead to decreased cell viability due to osmotic shock.

7. Slowly add 2 mL of complete Neurobasal™ Plus Medium to the tube (for a total suspension volume of 4 mL).

   Mix the suspension very gently with the 1-mL pipette without creating any air bubbles.

8. To a microcentrifuge tube containing 10 µL of 0.4% Trypan blue, add 10 µL of the cell suspension using a pre-rinsed tip. Mix by gently tapping the tube. Determine the viable cell density using a manual (i.e. hemocytometer) counting method.

   Do not centrifuge the cells as they are extremely fragile upon recovery from cryopreservation.

   IMPORTANT! Rinse each pipette tip and vial with complete Neurobasal™ Plus Medium before using it for cell suspension to prevent the cells from sticking to the plastic.

9. Plate ~1×10^5 live cells per well in a poly-D-lysine-coated (4.5 µg/cm^2) 48-well plate.

   Dilute the cell suspension to 500 µL per well by adding complete Neurobasal™ Plus Medium.

10. Incubate the cells at 36°C to 38°C in a humidified atmosphere of 5% CO^2 in air.

11. After 4–24 hours of incubation, aspirate half of the medium from each well and replace with fresh medium.

    Return the cells to the incubator.

12. Feed the cells every third day by aspirating half of the medium from each well and replacing it with fresh medium.

    IMPORTANT! Do not expose neurons to air at any time.

Use immunochemistry for detection of primary rat neuronal cells

1. Plate the cells on a poly-D-lysine-coated (4.5 µg/cm^2) 4-chamber slide by seeding at 2×10^5 live cells per chamber in 1 mL of medium.

2. Incubate the cells at 36°C to 38°C in a humidified atmosphere of 5% CO_2 in air.

3. After 24 hours of incubation, aspirate half of the medium from each well and replace with fresh medium.

   Return the cells to the incubator.

4. Feed the cells every third day by aspirating half of the medium from each well and replacing it with fresh medium.

5. When you are ready to perform an immunocytochemistry procedure, aspirate the supernatant and rinse the cells twice with DPBS, calcium, magnesium.

6. Fix the cells with 4% paraformaldehyde for 20 minutes.

7. Rinse the cells three times with DPBS, calcium, magnesium.

8. Permeabilize the cells with 0.3% Triton™ X-100 (diluted in DPBS, calcium, magnesium) for 5 minutes at room temperature.

9. Rinse the cells three times with DPBS, calcium, magnesium.

10. Incubate the cells coated with 5% goat serum solution diluted in DPBS, calcium, magnesium for 60 minutes at room temperature.

11. Incubate the cells coated with the primary antibody (Mouse anti-MAP2; 10 µg/mL; and/or Rabbit anti-GFAP, 4 µg/mL) diluted in 5% goat serum solution at 2°C –8°C overnight.

12. Rinse the cells three times with DPBS, calcium, magnesium.

13. Incubate the cells with the secondary antibody (Alexa Fluor™ 488 goat anti-mouse IgG (H+L), 10 µg/mL, and/or Alexa Fluor™ 594 goat anti-mouse IgG (H+L), 10 µg/mL, diluted in 5% goat serum solution for 60 minutes at room temperature.

14. Rinse the cells three times with DPBS, calcium, magnesium.

15. Stain the cells with DAPI solution (3 ng/mL) for 10 minutes.

16. Rinse the cells once with DPBS, calcium, magnesium.

17. Mount the cells with ProLong™ Gold Antifade Mountant.

Related products

Product	Cat. No.
Neurobasal™ Plus Medium	A3582901
B-27™ Plus Supplement (50X)	A3582801
GlutaMAX™ I Supplement (100X)	35050
DPBS, calcium, magnesium	14040
Goat Serum	16210-064
Mouse anti-MAP2	131500
Rabbit anti-GFAP	080063
Alexa Fluor™ 488 goat anti-mouse IgG	A11029
Alexa Fluor™ 594 goat anti-mouse IgG	A11037
(DAPI) 4', 6-diamidino-2-phenylindole, dihydrochloride	D3571
ProLong™ Gold Antifade Mountant	P36930

Limited product warranty

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