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# Primary Rat Cortex and Hippocampus Neurons

## 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.

| Product | Catalog no. | Amount | Storage |
|---|---|---|---|
| Primary Rat Cortex Neurons, 1x10$^6$ viable cells/vial (RCN 1M) | A10840-01 | 1 mL | Store in liquid nitrogen |
| Primary Rat Cortex Neurons, 4x10$^6$ viable cells/vial (RCN 4M) | A10840-02 | | |
| Primary Rat Hippocampus Neurons, 1x10$^6$ viable cells/vial (RHN 1M) | A10841-01 | | |

## Product use

For Research Use Only. Not for use in diagnostic procedures.

## Important information

The following procedures are designed for neuronal cells grown in Neurobasal®/B27® 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).

## Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Culture conditions

**Media:** Complete Neurobasal® 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 media

We recommend using Neurobasal® Medium (Cat. no. 21103) for primary rat neuron cultures. Add GlutaMAX™-I Supplement (Cat. no. 35050) and B-27® Supplement (Cat. no. 17504) to Neurobasal® Medium prior to use to prepare complete Neurobasal® Medium:

1. Aseptically add 200 mM GlutaMAX™-I Supplement to a final concentration of 0.5 mM (2.5 mL/L) to the medium before use.
2. Aseptically add 50X B-27® Supplement to a final concentration of 2% (v/v) (20 mL/L) to the medium before use.

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

## Recovery and culture of primary rat neurons

Note: **Do not vortex cells at any time during this procedure.**

1. Rinse a 15-mL conical culture tube with pre-warmed (37°C) complete Neurobasal®/B-27® 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 retighten cap.

   Note: Thaw one vial at a time. Transfer the vial immediately from liquid nitrogen storage to a 37ºC 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 P-1000 pipette tip with complete Neurobasal®/B-27® 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®/B-27® 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®/B-27® medium to the tube (for a total suspension volume of 4 mL). Mix the suspension very gently with the P-1000 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.

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

   IMPORTANT! Rinse each pipette tip and vial with complete Neurobasal®/B-27® 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®/B-27® medium.
10. Incubate the cells at 36°C –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.

Note: **Do not expose neurons to air at any time.**

## Immunocytochemistry 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 –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 with $Ca^{2+}$ and $Mg^{2+}$ (Cat. no. 14040).
6. Fix the cells with 4% paraformaldehyde for 20 minutes.
7. Rinse the cells three times with DPBS with $Ca^{2+}$ and $Mg^{2+}$.
8. Permeabilize the cells with 0.3% Triton®-X (diluted in DPBS with $Ca^{2+}$ and $Mg^{2+}$) for 5 minutes at room temperature.
9. Rinse the cells three times with DPBS with $Ca^{2+}$ and $Mg^{2+}$.
10. Incubate the cells coated with 5% goat serum solution (Cat. no. 16210) diluted in DPBS with $Ca^{2+}$ and $Mg^{2+}$ for 60 minutes at room temperature.
11. Incubate the cells coated with the primary antibody (Mouse anti-MAP2; 10 µg/mL; Cat. no. 131500 and/or Rabbit anti-GFAP, 4 µg/mL, Cat. no. 080063) diluted in 5% goat serum solution at 2°C –8°C overnight.
12. Rinse the cells three times with DPBS with $Ca^{2+}$ and $Mg^{2+}$.
13. Incubate the cells with the secondary antibody (Alexa Fluor® 488 goat-anti mouse (H+L), 10 µg/mL, Cat. no. A11029 and/or Alexa Fluor® 594 goat-anti rabbit (H+L), 10 µg/mL, Cat. no. A11037) diluted in 5% goat serum solution for 60 minutes at room temperature.
14. Rinse the cells three times with DPBS with $Ca^{2+}$ and $Mg^{2+}$.
15. Stain the cells with DAPI solution (3 ng/mL) for 10 minutes.
16. Rinse the cells once with DPBS with $Ca^{2+}$ and $Mg^{2+}$.
17. Mount the cells with Prolong® Gold anti-fade reagent (Cat. no. P36930).

## Related products

| Product | Cat. no. |
|---|---|
| Neurobasal® Medium (1X), liquid | 21103 |
| B-27® Serum-Free Supplement (50X) | 17504 |
| GlutaMAX™-I (100X) | 35050 |
| Dulbecco's Phosphate Buffered Saline (DPBS) with calcium, magnesium (1X), liquid | 14040 |
| Goat Serum | 16210 |
| Mouse anti-MAP2 | 131500 |
| Alexa Fluor® 488 goat anti—mouse IgG | A11029 |
| Alexa Fluor® 594 goat anti—rabbit IgG | A11037 |
| 4′, 6-diamidino-2-phenylindole, dilactate (DAPI) | D3571 |
| ProLong® Gold antifade reagent | P36930 |

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