Primary mouse cortex and hippocampus neurons

Catalog Numbers A15585, A15586, and A15587

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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**.

Description

Gibco™ Primary Mouse Cortex and Hippocampus Neurons are isolated from day-17 C57BL/6 mouse embryos and cryopreserved in a medium containing DMSO. Gibco™ Primary Mouse Neurons are the flexible, ready-to-use, and quality alternative to freshly isolated neurons.

Product	Catalog No.	Amount	Storage
Primary Mouse Cortex Neurons, 1 × 10^6 Viable Cells/vial (MCN 1M)	A15585	1 mL	Store in Liquid Nitrogen, vapor-phase
Primary Mouse Cortex Neurons, 4 × 10^6 Viable Cells/vial (MCN 4M)	A15586	1 mL	
Primary Mouse Hippocampus Neurons,1 × 10^6 Viable Cells/vial (MHN 1M)	A15587	1 mL	

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.

Culture conditions

Media: Complete Neurobasal™ Medium

Cell Lines: Primary Mouse Cortex and Hippocampus Neurons

Culture Type: Adherent

Recommended Substrate: Poly-D-Lysine at 4.5 µg/cm^2

Temperature Range: 36℃ to 38℃

Incubator Atmosphere: Humidified atmosphere of 5% CO_2 in air

Prepare media

Neurobasal™ Medium (Cat. no. 21103) is recommended for primary mouse neuron cultures. Complete Neurobasal™ Medium requires supplementation with GlutaMAX™-I Supplement (Cat. no. 35050) and B-27™ Supplement (Cat. no. 17504) 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 mouse hippocampus neuron cultures, the complete Neurobasal™ medium (prepared as described above) requires additional supplementation with 25 µM L-Glutamate up to day 4 in culture.

Recovery™ and culture of primary mouse neurons

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

1. Rinse a 50-mL conical culture tube with pre-warmed (37℃) complete Neurobasal™/B-27™ medium and leave it in the cell culture hood prior to thawing the cells.
2. If removing 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 37℃ 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. (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 50-mL tube (from Step 1 on page 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 50-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.

9. It is important to 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.

10. Plate ~0.5×10^5 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.

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

12. After 4 to 24 hours of incubation, aspirate half of the medium from each well and replace with fresh medium. Return the cells to the incubator.

13. 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 mouse neuronal cells

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

2. Incubate the cells at 36–38°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 ready to perform 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 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. 13-1500 and/or Rabbit anti-GFAP, 4 µg/mL, Cat. no. 08-0063) diluted in 5% goat serum solution at 2–8°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. A-11029 and/or Alexa Fluor™ 594 goat-anti rabbit (H+L), 10 µg/mL, Cat. no. A-11037) 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

Product	Cat. No.
Mouse anti-MAP2	13-1500
Rabbit anti-GFAP	A-11037
Alexa Fluor™ 488 goat anti—mouse IgG	A-11029
Alexa Fluor™ 594 goat anti—rabbit IgG	A-11037
4´, 6-diamidino-2-phenylindole, dilactate (DAPI)	D36930
ProLong™ Gold antifade reagent	P36930

Limited product warranty

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