B-27™ Plus Neuronal Culture System

Primary Neuron Applications

Catalog Numbers A3653401, A3582801, and A3582901

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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

The Gibco™ B-27™ Plus Neuronal Culture System is comprised of B-27™ Plus Supplement (50X) and Neurobasal™ Plus Medium. This system represents an evolution of the neuronal cell culture products, B-27™ Supplement and Neurobasal™ Medium, that is designed to provide maximum *in vitro* survival, improved maturation, and functionality of primary rodent neurons.

Contents and storage

Table 1 B-27™ Plus Neuronal Culture System, Cat. No. A3653401

Contents	Cat. No.	Amount	Storage	Shelf life[1]
Neurobasal™ Plus Medium	A3582901	500 mL	2°C to 8°C; Protect from light.	12 months
B-27™ Plus Supplement (50X)	A3582801	10 mL	–20°C to –5°C; Protect from light.	

[1] Shelf-Life duration is determined from Date of Manufacture.

Use

Maintenance and maturation of primary rat and mouse neurons.

Prepare complete Neurobasal™ Plus Medium

1. Thaw B-27™ Plus Supplement (50X) overnight at 4°C. Aseptically add 2% B-27™ Plus Supplement (50X) (20 mL/L) to the Neurobasal™ Plus Medium before use.

   Note: Remaining B-27™ Plus Supplement (50X) may be aliquoted into working volumes and stored at –20°C to –5°C. Thaw aliquots as needed. Do not freeze-thaw B-27™ Plus Supplement (50X) more than twice. Once thawed do not leave thawed supplement at 4°C for more than two weeks.

2. Aseptically add GlutaMAX™ I Supplement to 0.5 mM concentration (2.5 mL/L) to the medium before use.

   Note: Unlike Neurobasal™ Medium, Neurobasal™ Plus Medium contains 0.5 mM GlutaMAX™ I Supplement; however we have tested supplementing additional GlutaMAX™ I Supplement up to 2 mM final concentration with no detrimental effects on neuronal survival.

3. Pre-warm complete Neurobasal™ Plus Medium at 37°C in a water bath before using.

Cell culture procedure

The following procedure has been tested on freshly isolated embryonic and neonatal rodent neurons as well as cryopreserved neurons, including our Gibco™ Primary Rat Cortex Neurons, Gibco™ Primary Rat Hippocampus Neurons, Gibco™ Primary Mouse Cortex Neurons, Gibco™ Primary Mouse Hippocampus Neurons.

Coat culture plates with Poly-D-Lysine

1. Dilute the Poly-D-Lysine solution in sterile DPBS to prepare a 50 µg/mL working solution.

2. Coat the surface of the culture vessel with the working solution of Poly-D-Lysine (e.g. 50 µL/well of a 96-well plate).

3. Incubate the culture vessel at room temperature for 1 hour.

4. Remove the Poly-D-Lysine solution and rinse culture surface 3 times with sterile distilled water (e.g. 100 µL/well of a 96-well plate).

   Make sure to rinse the culture vessel thoroughly as excess Poly-D-Lysine solution can be toxic to the cells.

5. Remove distilled water and leave the coated culture vessel uncovered in the laminar hood to dry.

   The culture surface will be fully dry after 2 hours.

   Plates can be used immediately once dry or can be stored dry at 4°C. For storage at 4°C, tightly wrap the vessel with Parafilm™ film and use within one week of coating.

Culture neurons

1. Isolate primary neurons according to standard laboratory procedures or thaw cryopreserved neurons according to instructions supplied with the cells. See "Recover and culture cryopreserved neurons".

2. Plate cells on Poly-D-Lysine coated vessel using pre-warmed (37°C) complete Neurobasal™ Plus Medium (prepared as described in previous section) at density recommended in Table 2.

   Table 2 Seeding density

Source	Seeding density		
	Low (cells/cm^2)	Medium (cells/cm^2)	MEA Application High (cells/drop)
Rat	20,000	60,000	80,000
Mouse	30,000	60,000	60,000
Feeding schedule: (Suggested 1/2 volume change per feed)	1–2 times weekly	Every 3–4 days	Every 2–3 days

3. Incubate the culture dish at 37°C in a humidified atmosphere of 5% CO_2.

4. Four days after plating, feed the cultures by aspirating half of the medium from each well and replacing with same volume of fresh medium. Repeat every 2 to 3 days thereafter.

Recover and culture cryopreserved neurons procedure

Procedural guidelines

- Primary neuronal cells will adhere to bare pipette; to maximize cell recovery and yield we recommend pre-rinsing all plastic and glassware with complete medium before use.
- Do not vortex or centrifuge cells at any time during this procedure as cells are extremely fragile upon recovery from cryopreservation.
- Thaw one vial of neuronal cells at a time.
- Transfer cryovial from liquid nitrogen storage to 37°C water bath minimizing handling time.
- Dry ice can be used to transport the vials from liquid nitrogen to the water bath.

Recover and culture cryopreserved neurons

1. Rinse a sterile 15-mL conical culture tube with complete Neurobasal™ Plus Medium and leave in the hood prior to thawing cells.

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

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

   Remove from water bath when only one tiny ice crystal is left; the vial should still be cold to the touch.

4. Transfer the vial into the hood and disinfect with 70% isopropyl alcohol.

   Collect the liquid to the bottom of the vial by gently tapping the vial on the hood's surface.

5. Use a pre-rinsed 1-mL pipette tip to very gently transfer the cells to the pre-rinsed 15-mL conical tube.

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

   Mix by gentle swirling after each drop. Do not add the full amount of media to the tube at once. This may lead to decreased cell viability due to osmotic shock.

7. Slowly (dropwise) add an additional 2 mL of pre-warmed complete Neurobasal™ Plus Medium to the tube (for a total suspension volume of 4 mL).

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

8. Add 10 µL of cell suspension to a microcentrifuge tube containing 10 µL of 0.4% Trypan blue, using a pre-rinsed tip.

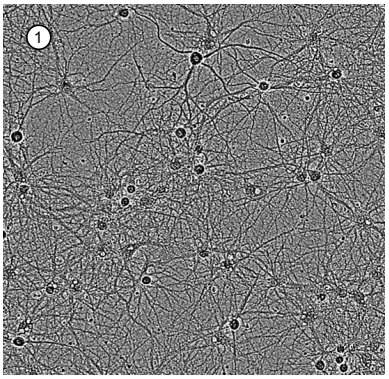
   Mix only by gently tapping the tube. Determine the viable cell density using a manual (i.e., hemocytometer) counting method. The viability of thawed cells should be >50%.

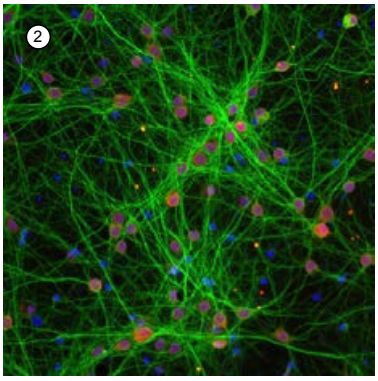
9. Seed cells in Poly-D-Lysine coated 96-well plate at densities recommended in Table 2.

10. Incubate the culture plate at 37°C in a humidified atmosphere of 5% CO_2.

11. After 4–24 hours of incubation, aspirate half of the medium and replace with same volume of fresh medium. Return the plate to the incubator. Repeat every 2–3 days thereafter.

Examples of primary mouse and rat neuronal cultures





① Phase contrast image of primary mouse cortical neurons cultured at day 21 in B-27™ Plus Neuronal Culture System.

② Primary Rat cortical neurons maintained in B-27™ Plus Neuronal Culture System for 35 days and stained with neuronal markers HuC/HuD (red), MAP2 (green). Cell nuclei were counter stained with DAPI (blue).

Troubleshooting

Observation	Possible cause	Recommended action
Primary rat and mouse neuronal cultures have undesired number of glial cells	The presence of glial cells can be due to mixed cultures which depend on age of animals at the time of neurons isolation, isolation technique and/or initial plating density.	Include CultureOne™ Supplement at 1X concentration at the time of plating cells to eliminate glial cells. Refer to *CultureOne™ Supplement (100X) User Guide* (Cat. No. A3320201).
		Delay the addition of CultureOne™ Supplement to days 2, 4, 6, or 8, to achieve desired levels of glial cells.
There are not many cells attached	Uneven substrate (Poly-D-Lysine) coating	Check if the substrate coating is even.
		Use freshly coated plate.
	Too few cells plated	Increase the cell plating density.
	Forgot to coat plate with Poly-D-Lysine	Remember to use Poly-D-Lysine coated plates (see protocol).
	Vibration affecting incubator	Eliminate or relocate sources of vibration such as centrifuges and shakers.
The media has turned yellow	Improved cell survival leads to greater media consumption.	More frequent media changes.
		Reduce seeding density of original number of cells.
	Serum or growth factors were added to the media	Don't add serum. B-27™ Plus Neuronal Culture System has been optimized to use without added serum or growth factors.
Primary rat and mouse neuronal cultures show clumped morphology	Problem with substrate coating	Check if the substrate coating is even.
Neurons suddenly die after five or more days in culture	Maturing neurons are more susceptible to excitotoxicity.	Supplement media only with GlutaMAX™ I Supplement. Do not use glutamine or non-essential amino acids.

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Product	Catalog No.
Reagents	
GlutaMAX™ I Supplement (100X), liquid	35050
CultureOne™ Supplement	A3320201
Poly-D-Lysine	A3890401
DPBS, calcium, magnesium	14040
DPBS, no calcium, no magnesium	14190
Trypan Blue Stain	15250
Image-iT™ Fixative Solution (4% formaldehyde, methanol-free)	FB002
Goat Serum	16210-064
Triton™ X-100	HFH100
Primary neurons	
Primary Rat Cortex Neurons: 1 × 10^6 viable cells/vial, 4 × 10^6 viable cells/vial	A1084001, A1084002
Primary Rat Hippocampus Neurons: 1 × 10^6 viable cells/vial	A10841
Primary Mouse Cortex Neurons : 1 × 10^6 viable cells/vial, 4 × 10^6 viable cells/vial	A15585, A15586
Primary Mouse Hippocampus Neurons: 1 × 10^6 viable cells/vial	A15587
Antibodies	
HuC/HuD Monoclonal Antibody	A-21271
MAP2 Polyclonal Antibody	PA5-17646
Goat anti-mouse IgG (H+L) Highly cross-Adsorbed Secondary Antibody, Alexa Fluor 488	A-11029
Goat anti-mouse IgG (H+L) Highly cross-Adsorbed Secondary Antibody, Alexa Fluor 594	A-11037
Instruments and consumables	
Countess™ II Automated Cell Counter	AMQAX1000
Nunc™ cell culture treated plates and dishes	167008, 165305, 150687, 142475, 150628, 140675, or 176740

Limited product warranty

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