B-27™ Supplement (50X)

Catalog Numbers 17504044, 17504001

Pub. No. MAN0007313 **Rev.** 3.0

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

Gibco™ B-27™ Supplement is an optimized serum-free supplement used to support the growth and viability of embryonic, post-natal, adult, hippocampal and other central nervous system (CNS) neurons. B-27™ Supplement is provided as a 50X liquid and is intended to be used with Neurobasal™ Medium or Neurobasal™-A Medium for cell culture of nearly pure populations (<0.5% Glial cell) of neuronal cells without the need for an astrocyte feeder layer. B-27™ Supplement includes a cocktail of antioxidants to reduce reactive oxygen damage.

Contents and storage

Contents	Cat. No.	Amount	Storage	Shelf life[1]
B-27™ Supplement (50X)	17504044	10 mL	–20°C to –5°C; Protect from light	12 months
	17504001	100 mL		

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

Use

- Use B-27™ Supplement to supplement Neurobasal™ Medium for optimal viability and long-term survival of pre-natal and embryonic neuronal cells.
- Use B-27™ Supplement to supplement Neurobasal™-A Medium for optimal viability and long-term survival of post-natal and adult brain neuronal cells.
- B-27™ Supplement when used as a supplement to Neurobasal™ Medium is effective for the culturing of tumor cell lines of neuronal origin.
- B-27™ Supplement when used as a supplement to DMEM/F-12, GlutaMAX™ Supplement mixture has been demonstrated to support the expansion of EGF-responsive precursor cells from rat embryonic striatum and mesencephalon.

Prepare complete Neurobasal™ Medium

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

   Note: Remaining B-27™ 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™ Supplement (50X) more than twice. Once thawed do not leave thawed supplement at 4°C for more than two weeks.
3. Aseptically add GlutaMAX™ Supplement to 0.5 mM concentration (2.5 mL/L) to the Neurobasal™ Medium before use.

   Note: For primary rat hippocampus neuron cultures, the complete Neurobasal™ Medium requires additional supplementation with 25 µM L-glutamate up to the fourth day in culture.

   Once supplemented, the complete Neurobasal™ Medium is stable for up to one week when stored in the dark at 2°C to 8°C.

Cell culture procedure

This procedure has been tested on freshly isolated 18-day gestation rat hippocampal and cortical neurons, Gibco™ Primary Rat Cortex Neurons, Gibco™ Primary Rat Hippocampus Neurons, and neuroblastoma cell lines.

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 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 cells

1. Isolate primary rat neurons or thaw cryopreserved primary rat neurons according to standard laboratory procedure or instructions supplied with the cells (See "Recover and culture cryopreserved neurons").
2. Plate cells in pre-warmed (37°C) complete Neurobasal™ Medium (See "Prepare complete Neurobasal™ Medium") at a suggested density of 160 cells/mm^2, or another optimized density if required.

   Note: For hippocampal neurons, use the complete medium supplemented with 25 µM L-Glutamate, see "Prepare complete Neurobasal™ Medium".
3. Incubate the culture dish at 36°C to 38°C in a humidified atmosphere of 5% CO_2 (in air is acceptable but 9% oxygen with 5% CO_2 is preferable).
4. 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.

Culture type conditions

Use the following conditions based on culture type.

- **Non-hippocampal cultures**: 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 three days thereafter.
- **Hippocampal cultures**: Three days after plating, replace half of the medium with complete medium without L-Glutamate. Repeat every three days thereafter.

  Note: Improved long-term survival of hippocampal neurons may be obtained by the addition of 25 µM 2-mercaptoethanol.

Isolate primary fetal neurons

This procedure is recommended for cultured 18-day embryonic rat hippocampal or cortical neurons.

1. Dissect cortex or hippocampi pairs from rat embryos at Day 18 of gestation.
2. Collect all the tissue in a conical tube containing complete Hibernate™ E Medium; see *Hibernate™-A and Hibernate™-B User Guide* (Pub. No. MAN0007380).

   Leave the tissue in this tube (1 pair/2 mL) until all the dissections are completed.
3. Let the tissue settle to the bottom of the tubes and then carefully remove supernatant leaving only the tissue covered by a minimum amount of medium.
4. Enzymatically digest the tissue in Hibernate™-E, minus Calcium (BrainBits LLC, Cat. No. HE-Ca) medium containing 2 mg/mL filter sterilized papain at 30°C for 30 minutes with gentle shaking of the tube every 5 minutes (2 pairs/mL).
5. Restore divalent cations with 2 volumes of complete Hibernate™ E Medium.
6. Allow non-dispersed tissue to settle for 2 minutes and then transfer the supernatant to a 15-mL tube and centrifuge for 5 minutes at 150 × *g*.
7. Gently resuspend the pellet in 1 mL complete Neurobasal™ Medium and take an aliquot (e.g., 10 µL) for cell counting.

   See "Recover and culture cryopreserved neurons", steps step 11–step 14.

   Note: Gibco™ Primary Rat Cortex Neurons and Primary Rat Hippocampus Neurons (isolated from Day-18 Fisher 344 rat embryos and cryopreserved in a medium containing 10% DMSO) are a quality ready-to-use alternative to freshly isolated neurons.

Recover and culture cryopreserved neurons

1. Rinse a sterile 15-mL conical culture tube with complete Neurobasal™ 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 retighten 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 (vial should still be cold to the touch).
4. Transfer the vial into the hood and disinfect with 70% isopropyl alcohol.
5. Collect the liquid to the bottom of the vial by gently tapping the vial on the hood's surface.
6. Use a pre-rinsed 1-mL pipette tip to very gently transfer the cells to the pre-rinsed 15-mL conical tube.
7. Rinse the cryovial with 1 mL of pre-warmed complete Neurobasal™ Medium and extremely slowly add to the cells in the 15-mL tube at the rate of one drop per second.
8. 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.
9. Slowly (dropwise) add an additional 2 mL of prewarmed complete Neurobasal™ Medium to the tube (for a total suspension volume of 4 mL).
10. Mix the suspension very gently with 1-mL pipette without creating any air bubbles.
11. 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.
12. Determine the viable cell density using a manual (i.e., hemocytometer) counting method.

    The viability of thawed cells should be >50%.
13. Plate ~1 × 10^5 cells (see "Culture cells", step 2–step 3) (or desired cell density) per well in a Poly-D-Lysine coated 48-well plate (see "Coat culture plates with Poly-D-Lysine"). Dilute cell suspension to 500 µL per well by adding pre-warmed complete Neurobasal™ Medium.
14. See "Culture cells" to maintain neuronal cell cultures.
15. Incubate at 36°C to 38°C in a humidified atmosphere of 5% CO_2 (in air is acceptable but 9% oxygen with 5% CO_2 is preferable).

Cell lines

Some cell lines may require an initial attachment in 2% serum supplemented Neurobasal™ Medium. Serum-free complete Neurobasal™ Medium can then be added after incubation for 2 hours or overnight.

Subculture immortalized cell lines

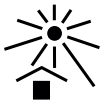
1. Aspirate spent media and wash cells with HBSS, no calcium, no magnesium, no phenol red.
2. Add sufficient Trypsin-EDTA (0.25%) to cover cell monolayer, aspirate excess Trypsin-EDTA (0.25%) solution.
3. Incubate for 2–4 minutes at 37°C; a strong tap to the vessel should detach cells from the substratum.
4. Add 5 mL HBSS, calcium, magnesium, no phenol red containing 0.05% Trypsin Inhibitor, Soybean to quench trypsin activity.
5. Transfer to a sterile 15-mL tube and centrifuge at 200 × *g* for 2 minutes at room temperature.
6. Aspirate supernatant and gently resuspend cell pellet in complete Neurobasal™ Medium.
7. Determine viable cell density using a Countess™ II Automated Cell Counter.
8. Dilute cells into Poly-D-Lysine coated culture vessels with complete Neurobasal™ Medium at ~160 cells/mm^2 or another user optimized density if required.

Related products

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

Item	Source
B-27™ Supplement (50X) minus antioxidants	10889
Neurobasal™ Medium	21103
Neurobasal™-A Medium	10888
GlutaMAX™ Supplement (100X)	35050
Hibernate™ E Medium	A12476
Poly-D-Lysine	A3890401
Primary Rat Cortex Neurons	A1084001
Primary Rat Hippocampus Neurons	A10841
Trypsin-EDTA (0.25%), phenol red	25200
Trypsin Inhibitor, Soybean	17075
HBSS, calcium, magnesium, no phenol red	14025
HBSS, no calcium, no magnesium, no phenol red	14175
2-Mercaptoethanol	21985
Countess™ II Automated Cell Counter	AMQAX1000
Trypan Blue Stain	15250

Explanation of symbols

Symbol	Description	Symbol	Description	Symbol	Description
	Manufacturer		Catalog number		Batch code
	Use by		Temperature limitation		Keep away from light
	Sterilized using aseptic processing techniques		Consult instructions for use		Caution, consult accompanying documents

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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