

# B-27<sup>®</sup> Supplement (50X)

## Description

B-27<sup>®</sup> Supplement is an optimized serum-free supplement used to support the growth and viability of embryonic, post-natal, and adult, hippocampal and other central nervous system (CNS) neurons. B-27<sup>®</sup> Supplement is provided as a 50X liquid and is intended to be used with Neurobasal<sup>®</sup> Medium or Neurobasal<sup>®</sup>-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<sup>®</sup> Supplement includes a cocktail of antioxidants to reduce reactive oxygen damage.

Product	Catalog No.	Amount	Storage	Shelf Life*
B-27 <sup>®</sup> Supplement (50X), liquid	17504-044	10 mL	-20°C to -5°C; Protect from light	12 months
	17504-001	100 mL		

\* Shelf Life duration is determined from Date of Manufacture.

## Product Use

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

## Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions.

Wear appropriate protective eyewear, clothing, and gloves.

## Prepare Medium

1. Neurobasal<sup>®</sup> Medium is recommended for primary neuronal cultures. Neurobasal<sup>®</sup> Medium requires supplementation with GlutaMAX<sup>™</sup>-I (200mM) and B-27<sup>®</sup> Supplement (50X) prior to use. Thaw B-27<sup>®</sup> Supplement overnight at 4°C.
  2. Aseptically add GlutaMAX<sup>™</sup>-I to 0.5 mM final concentration (2.5 mL/L) to the medium before use.
  3. Aseptically add 2% B-27<sup>®</sup> Supplement (20 mL/L) to the medium before use.
- Note:** Remaining B-27<sup>®</sup> Supplement may be aliquoted into working volumes and stored at -20°C to -5°C. Thaw aliquots as needed. Do not freeze-thaw B-27<sup>®</sup> Supplement more than twice.
4. For primary rat hippocampus neuron cultures, the complete Neurobasal<sup>®</sup> medium (prepared from the previous steps) requires additional supplementation with 25 μM L-Glutamate up to the fourth day in culture.

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

## Use

- Use B-27<sup>®</sup> Supplement to supplement Neurobasal<sup>®</sup> Medium for optimal viability and long-term survival of pre-natal and embryonic neuronal cells.
- Use B-27<sup>®</sup> Supplement to supplement Neurobasal<sup>®</sup>-A Medium for optimal viability and long-term survival of post-natal and adult brain neuronal cells.
- B-27<sup>®</sup> Supplement when used as a supplement to Neurobasal<sup>®</sup> Medium is effective for the viability of tumor cell lines of neuronal origin.
- B-27<sup>®</sup> Supplement when used as a supplement to DMEM/F12 mixture has been demonstrated to support the expansion of EGF-responsive precursor cells from rat embryonic striatum and mesencephalon.

## Cell Culture Procedure

The following procedure has been tested on freshly isolated 18-day gestation rat hippocampal and cortical neurons, Gibco<sup>®</sup> Primary Rat Cortex Neurons, Gibco<sup>®</sup> Primary Rat Hippocampus Neurons, and neuroblastoma cell lines.

1. Coat culture surface (German glass or cell culture grade plastics) with a sterile 0.05 mg/mL solution of cold poly-D-lysine in water at 0.15 mL/cm<sup>2</sup> surface area and incubate for 1 hour at ambient temperature.

2. Remove poly-D-lysine solution, and rinse twice with sterile distilled H<sub>2</sub>O. (Rinse thoroughly, since poly-D-lysine can be toxic to the cells). Leave the plates uncovered in the hood until the wells are completely dry. Plates can be used immediately once dry or can be stored dry at 4°C for up to 2 weeks.
3. Isolate primary rat neurons or thaw cryopreserved primary rat neurons according to standard laboratory procedure or instructions supplied with the cells (See **Recovery and Culturing of Cryopreserved Neurons**).
4. Plate cells in pre-warmed (37°C) complete Neurobasal<sup>®</sup> Media (prepared as described above) at a suggested density of 160 cells/mm<sup>2</sup>, or another optimized density if required.  
**Note:** For hippocampal neurons, use the complete medium supplemented with 25 μM L-Glutamate, see **Prepare Medium**.
5. Incubate the culture dish at 36°C to 38°C in a humidified atmosphere of 5% CO<sub>2</sub> (in air is acceptable but 9% oxygen with 5% CO<sub>2</sub> is preferable).
6. 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.
7. **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

1. The following procedure is recommended for cultured 18-day embryonic rat hippocampal or cortical neurons.
2. Dissect cortex or hippocampi pairs from rat embryos at Day 18 of gestation (E18).
3. Collect all the tissue in a conical tube containing Hibernate-E complete medium. Leave the tissue in this tube (1 pair/2 mL) until all the dissections are completed.
4. 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.
5. Enzymatically digest the tissue in Hibernate-E, without Ca<sup>2+</sup> (BrainBits<sup>®</sup> 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).
6. Restore divalent cations with 2 volumes of Hibernate-E complete medium.
7. 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.

- Gently resuspend the pellet in 1 mL complete Neurobasal<sup>®</sup> medium and take an aliquot (e.g., 10  $\mu$ L) for cell counting. Proceed to **Recovery and Culturing of Cryopreserved Neurons** steps 8–10.

**Note:** Gibco<sup>®</sup> Primary Rat Cortex and 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.

(See **Recovery and Culturing of Cryopreserved Neurons**).

## Recovery and Culturing of Cryopreserved Neurons

**Important:** Primary neuronal cells will adhere to bare plastic and glassware; 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. We recommend thawing one vial at a time. Transfer cryovial from liquid nitrogen storage to 37°C water bath minimizing handling time. A small amount of liquid nitrogen in an ice bucket can be used to transport the vials from liquid nitrogen to the water bath.

- Rinse a sterile 15-mL conical culture tube with complete Neurobasal<sup>®</sup> Media and leave in the hood prior to thawing cells.
- If removing vial from liquid nitrogen storage, twist cap slightly to release pressure and then retighten cap.
- 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).
- 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.
- Use a pre-rinsed P-1000 pipette tip to very gently transfer the cells to the pre-rinsed 15-mL conical tube.
- Rinse the cryovial with 1 mL of pre-warmed complete Neurobasal<sup>®</sup> Media 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.
- Slowly (dropwise) add an additional 2 mL of pre-warmed complete Neurobasal<sup>®</sup> Media to the tube (for a total suspension volume of 4 mL). Mix the suspension very gently with P-1000 pipette without creating any air bubbles.
- Add 10  $\mu$ L of cell suspension to a microcentrifuge tube containing 10  $\mu$ L 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%.
- Plate  $\sim 1 \times 10^5$  cells (see **Cell Culture Procedure**, steps 4–5) (or desired cell density) per well in a poly-D-lysine coated 48-well plate (see **Cell Culture Procedure**, steps 1–2). Dilute cell suspension to 500  $\mu$ L per well by adding pre-warmed complete Neurobasal<sup>®</sup> Media.
- Follow **Cell Culture Procedure** steps 6–7 to maintain neuronal cell cultures. Incubate at 36°C to 38°C in a humidified atmosphere of 5% CO<sub>2</sub> (in air is acceptable but 9% oxygen with 5% CO<sub>2</sub> is preferable).

## Cell Lines

**Note:** Some cell lines may require an initial attachment in 2% serum-supplemented Neurobasal<sup>®</sup> Medium. Serum-free complete Neurobasal<sup>®</sup> Medium can then be added after incubation for 2 hours or overnight.

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).

For further assistance, email [techsupport@lifetech.com](mailto:techsupport@lifetech.com)

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## Subculture Immortalized Cell Lines










- Aspirate spent media and wash cells with Hank's Balanced Salt solution (HBSS) without calcium and magnesium.
- Add sufficient 0.25 % trypsin/1.0 mM EDTA to cover cell monolayer, aspirate excess trypsin/EDTA solution.
- Incubate for 2–4 minutes at 37°C; a strong tap to the vessel should detach cells from the substratum.
- Add 5 mL HBSS with calcium and magnesium containing 0.05% Soybean Trypsin Inhibitor to quench Trypsin activity.
- Transfer to a sterile 15-mL tube and centrifuge at 200  $\times$  g for 2 minutes at room temperature.
- Aspirate supernatant and gently resuspend cell pellet in complete Neurobasal<sup>®</sup> Medium. Determine viable cell density using a Countess<sup>®</sup> Automated Cell Counter.
- Dilute cells into poly-D-lysine coated culture vessels with complete Neurobasal<sup>®</sup> Medium at  $\sim 160$  cells/mm<sup>2</sup> or another user optimized density if required.

## Related Products

Product	Catalog No.
B-27 <sup>®</sup> Supplement Minus AO (50X), liquid	10889
Neurobasal <sup>®</sup> Medium (1X), liquid	21103
Neurobasal <sup>®</sup> -A Medium (1X), liquid	10888
GlutaMAX <sup>™</sup> -I (100X), liquid	35050
Hibernate E	A12476
Primary Rat Cortex Neurons, 1 $\times$ 10 <sup>6</sup> viable cells/vial 4 $\times$ 10 <sup>6</sup> viable cells/vial	A10840-01 A10840-02
Primary Rat Hippocampus Neurons, 1 $\times$ 10 <sup>6</sup> viable cells/vial	A10841
0.25% Trypsin-EDTA (1X), 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 (1000X), liquid	21985
Countess <sup>®</sup> Automated Cell Counter	C10227
Trypan Blue Stain	15250

## Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

				
Use By:	Manufacturer	Batch code	Keep away from light	Temperature Limitation
				
Catalog number	Consult instructions for use	Caution, consult accompanying documents	Sterilized using aseptic processing techniques	

## Limited Product Warranty

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