gibco by life technologies

B-27® Supplement Minus AO (50X)

Description

B-27® Supplement Minus Antioxidants (AO) is a customized B-27® Serum-Free Supplement without 5 antioxidants (vitamin E, vitamin E acetate, superoxide dismutase, catalase, and glutathione). B-27® Supplement Minus AO is a serum-free supplement designed for the study of the role of free-radicals in aging, toxicity, apoptosis and chronic neurologic diseases. B-27® Supplement Minus AO 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.

Product	Catalog no.	Amount	Storage	Shelf life*
B-27® Supplement Minus AO (50X), liquid	10889-038	10 mL	–20°C to –5°C; Protect from light	12 months

* Shelf life duration is determined from Date of Manufacture.

Product use

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

Important information

- B-27® Supplement Minus AO is identical to B-27® Supplement except for the removal of antioxidant components, vitamin E, vitamin E acetate, superoxide dismutase, catalase, and glutathione from the formula.
- Once antioxidants are added back product effects can be compared to a control complete B-27® Supplement.

Safety information

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

Caution: Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV, and HB_sAg. Handle in accordance with established bio-safety practices.

Use

- Use B-27® Supplement Minus AO to supplement Neurobasal® Medium for optimal viability and long-term survival of pre-natal and embryonic neuronal cells.
- Use B-27® Supplement Minus AO to supplement Neurobasal®-A Medium for optimal viability and long-term survival of post-natal and adult brain neuronal cells.

Prepare medium

Neurobasal® Medium is recommended for primary neuronal cultures. Neurobasal® Medium requires supplementation with GlutaMAX™-I (200mM) and B-27® Supplement Minus AO (50X) prior to use. Thaw B-27® Supplement Minus AO overnight at 4°C.

1. Aseptically add GlutaMAX™-I to 0.5 mM final concentration (2.5 mL/L) to the medium before use.
2. Aseptically add 2% B-27® Supplement Minus AO (20 mL/L) to the medium before use.
   Note: Remaining B-27® Supplement Minus AO 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 more than twice.
3. For primary rat hippocampus neuron cultures, the complete Neurobasal® 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® Medium is stable for up to one week when stored in the dark at 2°C to 8°C.

Cell culture procedure

The following 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.

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^2 surface area and incubate for 1 hour at ambient temperature.
2. Remove poly-D-lysine solution, and rinse twice with sterile distilled H_2O. (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® Media (prepared as described above) 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 medium**.
5. 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).
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

The following 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 (E18).
2. 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.
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, without Ca^{2+} (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 Hibernate-E complete 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. Proceed to **Recovery and culturing of cryopreserved neurons** steps 8–10.
   Note: Gibco® 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 glass-ware; 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.

1. Rinse a sterile 15-mL conical culture tube with complete Neurobasal® Media 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. Collect the liquid to the bottom of the vial by gently tapping the vial on the hood's surface.
5. Use a pre-rinsed P-1000 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® Media and extremely slowly add to the cells in the 15-mL tube at the rate 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® 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.
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. Plate ~1×10^5 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 µL per well by adding pre-warmed complete Neurobasal® Media.
10. 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_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 Hank's Balanced Salt solution (HBSS) without calcium and magnesium.
2. Add sufficient 0.25 % trypsin/1.0 mM EDTA to cover cell monolayer, aspirate excess trypsin/EDTA 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 with calcium and magnesium containing 0.05% Soybean Trypsin Inhibitor 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 the supernatant and gently resuspend cell pellet in complete Neurobasal® Medium. Determine viable cell density using a Countess® Automated Cell Counter.
7. 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

Product	Catalog no.
B-27® Supplement (50X), liquid	17504
Neurobasal® Medium (1X), liquid	21103
Neurobasal®-A Medium (1X), liquid	10888
GlutaMAX™-I (100X), liquid	35050
Hibernate E	A12476
Primary Rat Cortex Neurons, 1×10^6 viable cells/vial 4×10^6 viable cells/vial	 A10840-01 A10840-02
Primary Rat Hippocampus Neurons, 1×10^6 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® 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

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.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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For further assistance, email **techsupport@lifetech.com**