

B-27 Supplement (50X), Serum-Free

Description

TargetMol's B-27 Supplement (50X), Serum-free is a high-performance cell culture supplement specifically formulated for central nervous system (CNS) research. It effectively supports the growth, differentiation, and functional maintenance of neurons under serum-free conditions. This product is suitable for the in vitro culture of embryonic, postnatal, and adult hippocampal neurons as well as other CNS neurons. It is widely used in various culture systems, including adherent monolayers and floating neurospheres, making it an ideal choice for expanding neural progenitor cells and neural stem cells without inducing premature differentiation.

Supplied as a 50X concentrate, B-27 Supplement is recommended for use in combination with Neurobasal or Neurobasal-A media, significantly enhancing neuronal viability and long-term survival without the need for an astrocyte feeder layer. In addition, this supplement is suitable for culturing neurogenic tumor cell lines and has been broadly applied in the establishment and maintenance of organoid models derived from breast cancer, small intestine, stomach, and liver, demonstrating exceptional versatility across multiple research fields.

Product Features

1. Serum-free with optimized components to enhance experimental consistency.
2. Supports long-term culture of neurons, neural stem cells, and neural progenitor cells.
3. Applicable to both adherent and suspension culture systems.
4. Eliminates the need for an astrocyte feeder layer.
5. Compatible with organoid and neurogenic tumor model cultures.

Applications

Culture and maintenance of rat hippocampal neurons and various CNS neurons; expansion and neural differentiation of stem cells; viability maintenance of neurogenic tumor cell lines; optimization of long-term survival for embryonic and postnatal neurons; induction of neural and astrocytic differentiation from embryonic stem cells; establishment and culture of tumor organoids.

Instruction for Use

1. Product Dilution and Medium Preparation

B-27 Supplement (50X), Serum-free is supplied as a 50X concentrate and should be diluted 1:50 to a 1X working concentration prior to use. For example, to prepare 50 mL of complete medium, add 1 mL of B-27 Supplement to 49 mL of basal medium (such as Neurobasal or Neurobasal-A) and mix thoroughly.

Preparation of Complete Neuronal Medium:

- (1) Thaw the product slowly at 4°C.
- (2) Under sterile conditions, add 2% B-27 Supplement and 0.5 mM L-glutamine to the basal neuronal medium to prepare complete neuronal medium prior to use. Prepared complete medium can be stored protected from light at 2–8°C for up to one week.

Notes:

- a) L-Glutamine is unstable in aqueous solutions and its breakdown products can be toxic to cells; therefore, it should be added fresh during medium preparation.
- b) For primary hippocampal neuron culture, supplementing the complete neuronal medium with an additional 25 µM L-glutamic acid is recommended; omit L-glutamic acid during medium changes starting from day 4 of culture.
- c) Aliquot any remaining unused supplement into working volumes and store at -20°C to avoid repeated freeze-thaw cycles.

2. Neuronal Culture Protocol (Taking Fetal Rat Cortical Neurons as an Example)

- (1) Surface Coating Preparation:
 - a) Coat culture surfaces (e.g., glass coverslips or cell culture-grade plastic) with cold, sterile 0.05 mg/L poly-L-lysine aqueous solution at a volume of 0.15 mL/cm²; incubate overnight (16 h) at 37°C.
 - b) Aspirate the coating solution, rinse twice with sterile distilled water, and dry completely before use. Coated plates can be stored at 4°C for up to two weeks.
- (2) Cell Seeding and Culture:
 - a) Prepare freshly isolated primary neurons or thawed cryopreserved neurons for culture.
 - b) Pre-warm the complete neuronal medium to 37°C. A recommended seeding density is 2×10^5 cells/well for a 24-well plate, which can be adjusted based on experimental needs.
 - c) Incubate the seeded cells in a 37°C, 5% CO₂ incubator.
 - d) Perform a 50% medium change with fresh complete medium 16–24 h post-seeding, and perform half-volume medium changes every 2–3 days thereafter.

3. Isolation of Primary Neurons

Suitable for isolating hippocampal and cortical neurons from embryonic day 18 (E18) rat embryos:

- (1) Dissect cerebral cortex and bilateral hippocampal tissues from E18 embryos.
- (2) Transfer all tissue fragments into a conical tube containing pre-warmed complete medium and allow tissues to settle.
- (3) Carefully aspirate the supernatant, leaving a minimal layer of medium covering the tissue.
- (4) Add 2 mg/L filter-sterilized papain solution (prepared in calcium-free medium) and digest at 37°C for approximately 30 min, gently swirling the tube every 5 min. Use 2 mL of papain solution per pair of hippocampi.
- (5) Add two volumes of complete medium to restore divalent cation concentrations and stop enzymatic digestion.

- (6) Allow undissociated tissue fragments to settle for approximately 2 min, then transfer the supernatant to a 15 mL centrifuge tube and centrifuge at 150 x g for 5 min.
- (7) Resuspend the cell pellet in 1 mL of complete neuronal medium. Take 10 µL for cell counting and proceed according to Step 8 in "4. Thawing and Culture of Cryopreserved Neurons".

4. Thawing and Culture of Cryopreserved Neurons

Cryopreserved primary neurons are extremely sensitive to handling conditions. Cells are highly susceptible to damage during thawing; avoid vigorous shaking or high-speed centrifugation. It is recommended to thaw only one vial at a time and minimize the transfer time from liquid nitrogen to the 37°C water bath. To maximize cell attachment and recovery, pre-wet all plastic and glass labware surfaces with complete neuronal medium prior to use.

- (1) Pre-wet a sterile 15 mL conical tube with complete neuronal medium, discard the medium, and let it dry in a biosafety cabinet for use.
- (2) Retrieve the cryovial from liquid nitrogen storage, briefly loosen the cap to release built-up pressure, and retighten.
- (3) Rapidly thaw the cryovial in a 37°C water bath with gentle swirling for no more than 2 min. Remove the vial when a tiny ice crystal remains and the vial still feels cold.
- (4) Gently tap the vial on the benchtop to settle the liquid. Using a pre-wetted 1 mL sterile pipette tip, gently transfer the cell suspension into the prepared 15 mL conical tube.
- (5) Rinse the cryovial with 1 mL of pre-warmed complete neuronal medium, then slowly add it dropwise (approx. 1 drop per second) to the cell suspension in the 15 mL tube. Gently swirl the tube after each drop to avoid adding the entire volume at once.
- (6) Slowly add an additional 2 mL of pre-warmed complete medium to bring the total volume to 4 mL. Mix gently using a 1 mL pipette tip, taking care to avoid generating bubbles.
- (7) Take 10 µL of the cell suspension using a pre-wetted tip, mix with an equal volume of 0.4% trypan blue, and assess cell viability. Post-thaw viability should exceed 50%.
- (8) Seed cells into poly-L-lysine pre-coated 24-well plates at approximately 2×10^5 cells/well (or adjust according to experimental needs). Add pre-warmed complete medium to bring the final volume to 500 µL per well. Culture the plate in a 37°C, 5% CO₂ incubator.

Storage

Store at -20°C. Stable for 2 years.

Precautions

1. Thaw the product slowly at 4°C (overnight recommended) prior to use.
2. Mix thoroughly before pipetting to ensure homogenous distribution of components.
3. Maintain strict aseptic techniques during handling to prevent contamination.
4. Avoid prolonged exposure to room temperature or elevated temperatures to preserve product performance.
5. This product is for professional research use only. It is not intended for clinical diagnosis or treatment, food, or drug use, and should not be stored in residential areas.
6. For your safety and health, please wear a lab coat and disposable gloves during operations.

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