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by Thermo Fisher Scientific

B-27™ Supplement without Vitamin A (50X)

Description

B-27™ Supplement without Vitamin A is a serum-free, customized B-27™ Supplement without Vitamin A (retinyl acetate). The absence of vitamin A makes this formulation ideal for the cultivation of neural progenitor and stem cells, either as neurospheres in suspension or in adherent monolayer culture, without inducing differentiation. B-27™ Supplement without Vitamin A is provided as a 50X liquid and is intended to be used with Neurobasal™ or Neurobasal™-A media.

Product	Catalog no.	Amount	Storage	Shelf life*
B-27™ Supplement without Vitamin A (50X), liquid	12587-010 12587-001	10 mL 100 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 without Vitamin A does not contain retinyl acetate, a component of standard B-27™ Supplement, which can substitute for retinoic acid to stimulate differentiation of neural precursor/stem cells (NSCs).
- B-27™ Supplement without Vitamin A includes a cocktail of antioxidants to reduce reactive oxygen damage.

Safety information

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

Culture conditions

Media: Complete Neurobasal™-A or Neurobasal™ Media

Culture type: Suspension or Adherent

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 5% CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Culture vessels: Multiwell plates or T-flasks pre-treated as follows:

Suspension culture:

- Use pretreated "ultra low attachment" ULA cultureware (Costar™); alternatively, treat cultureware with anti-adhesive solution (TPP, poly (2-hydroxyethyl methacrylate).

Monolayer culture:

1. Incubate tissue cultureware with 15 µg/mL poly-L-ornithine overnight at 37°C.
2. Aspirate solution and wash twice with Dulbecco's Phosphate Buffered Saline (DPBS) without calcium or magnesium, allowing 20 minutes incubation at 37°C for each wash.
3. Incubate tissue cultureware with fibronectin (1 mg/L in DPBS without calcium or magnesium) for 1–24 hours at 37°C.
4. Aspirate solution and wash once with DPBS without calcium or magnesium. Dishes may be stored with DPBS without calcium or magnesium for 4 days at 37°C. Rinse with media before use.

Prepare complete medium

Neurobasal™-A Medium is recommended for the serum-free growth of NSCs; Neurobasal, DMEM//F12 and DMEM low glucose may also be used. Aseptic supplementation with Epidermal Growth Factor (EGF) and basic-Fibroblast Growth Factor (bFGF) in addition to B-27™ Supplement without Vitamin A is necessary to prevent spontaneous differentiation of NSCs in culture. Prepare a concentrated growth factor stock solution (500 µg/mL each in PBS with 0.1% bovine serum albumin), aliquot in working volumes and store at –20°C.

1. Aseptically add GlutaMAX™-I to 2–4 mM final concentration (10–20 mL/L) to Neurobasal™-A Medium before use.
2. Aseptically add 2% B-27™ Supplement without Vitamin A (20 mL/L) to the medium before use.

   Note: Remaining B-27™ Supplement without Vitamin A 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 without Vitamin A more than twice.

   Note: Once supplemented, the complete Neurobasal™-A Medium is stable for up to one week when stored in the dark at 2°C to 8°C.
3. Immediately before use thaw and aseptically add desired growth factors to 10–20 ng/mL.

Use

- B-27™ Supplement without Vitamin A when used as a supplement to Neurobasal™ (pre-natal/embryonic cells), Neurobasal™-A (post-natal/adult) or DMEM/F12 Medium supports the serum-free growth of Central Nervous System (CNS) and Peripheral Nervous System (PNS) embryonic and adult neural stem cells (NSCs) or neural precursor cells.
- B-27™ Supplement wihout Vitamin A promotes improved growth of neural precursor/stem cells (NSCs) as compared to ITS or N-2 Supplement and can also replace ITS and N-2 supplement (or variants) when differentiating ES cells (as embryoid bodies, EB) to nestin-positive neural precursor cells in culture.
- Areas of application include studies of gene function and neural development, neural differentiation and possibly for understanding the role of stem cells in replacement therapy for genetic and degenerative diseases.

Isolation and culture of Neural Stem Cells (NSCs)

The following culture conditions have been found effective with NSCs isolated from E-14 to E-18 (day-14 to 18 gestation) rat cortex, striatum, mesencephalon, thalamus, spinal cord or hippocampi.

1. Under sterile conditions embryonic CNS or PNS tissue from desired region are harvested into ice-cold Hanks' Balanced Salt Solution (HBSS) without calcium or magnesium in a 10 cm^2 sterile culture dish.
2. Transfer tissue, using a sterile siliconized glass Pasteur pipette, into a sterile 15-mL conical tube with 3 mL growth media for each tissue sample.
3. Dissociate tissue by gently triturating 20–30 times or until suspension looks cloudy and only very small fragments remain. Avoid foaming and bubbles. Stand tube upright for 1 minute to allow fragments to settle.
4. Using a new sterile siliconized glass Pasteur pipette, transfer the suspension to a new sterile 15-mL tube. Avoid transferring the undissociated fragments that have settled to the bottom of the tube.
5. Centrifuge cell suspension at 100 × *g* for 10 minutes. Discard supernatant. Flick the tube to disperse the cells in the pellet.
6. Resuspend the cells in 1–2 mL pre-warmed complete medium. Aliquot a small sample volume for use determining viable cell density with a Countess™ II Automated Cell Counter.

   Note: Viability should be approximately 70–80%.
7. Seed the cells at a density of 2.5 × 10^4–5 × 10^4 viable cells/cm^2 (adherent monolayer cultures) or 1 × 10^5 viable cells/mL (suspension cultures) in pre-warmed complete medium.

Subculture cells

Neurosphere suspension cultures.

1. Collect and transfer neurospheres into a sterile centrifuge tube when they have reached ~100 µm in diameter (2–6 days).
2. Centrifuge at 100 × *g* for 10 minutes, discard supernatant.
3. Resuspend neurospheres in 1 mL fresh pre-warmed complete medium and mechanically dissociate by triturating with a small-bore pipette tip. Avoid foaming and bubbles.
4. Determine total viable cell density using Countess™ II Automated Cell Counter.
5. Seed cells at 1 × 10^5 viable cells/mL in pre-warmed complete medium.

Monolayer cultures.

1. Passage cells when the culture is 50–70% confluent.
2. Detach cells by gentle scraping with a cell lifter.
3. Transfer cells to a sterile centrifuge tube. Centrifuge at 100 × *g* for 10 minutes and discard spent medium.
4. Resuspend the cell pellet in 1–2 mL pre-warmed complete medium. Determine viable cell density using a Countess™ II Automated Cell Counter.
5. Seed the cells at a density of 2.5 × 10^4–5 × 10^4 viable cells/cm^2 in pre-warmed complete medium.

   Note: Successful culture of NSCs requires seeding at low cell density (~ 5 × 10^4 cells/cm^2), complete absence of serum, frequent addition of fresh growth factors, absence of a strong adhesion substrate, and efficient dissociation of neurospheres to a single cell suspension without reducing viability due to cell disruption.

Differentiation of NSCs

Neural stem cells cultured continuously according to the above instructions can be promoted to spontaneously differentiate into neurons by removing the growth factors. This is achieved by replacing complete media with Neurobasal™ supplemented with 2% standard B-27™ Supplement and 2 mM GlutaMAX™-I, and plating the cells onto a strong adhesion substrate.

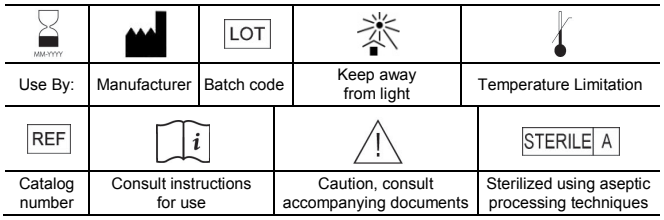
For more information on differentiating neural stem cells into neurons and glia, visit **www.thermofisher.com/neuroculture**.

Related products

Product	Catalog no.
Neurobasal™ Medium (1X), liquid	21103
Neurobasal™-A Medium (1X), liquid	10888
DMEM, low glucose, pyruvate, HEPES	12320
B-27™ Supplement (50X), liquid	17504
GlutaMAX™-I (100X), liquid	35050
EGF Recombinant Human	PHG0311
FGF-basic Recombinant Human	13256
Fibronectin Human, Plasma	33016
Insulin-Transferrin-Selenium-G Supplement (100X)	41400
N-2 Supplement (100X), liquid	17502
DPBS, no calcium, no magnesium	14190
HBSS, no calcium, no magnesium, no phenol red	14175
Countess™ II Automated Cell Counter	AMQAX1000
Countess™ II FL Automated Cell Counter	AMQAF1000
Trypan Blue Stain	15250

Explanation of symbols and warnings

The symbols present on the product label are explained below:



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