

B-27™ Supplement Xeno-Free minus Vitamin A (50X)

Catalog Number A3353501

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 **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](https://www.thermofisher.com/support).

Product description

Gibco™ B-27™ Supplement Xeno-Free minus Vitamin A is a customized B-27™ Supplement deficient in 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 Xeno-Free minus Vitamin A is provided as a 50X liquid and is intended to be used with CTS™ Neurobasal™ Medium or CTS™ Neurobasal™ -A Medium.

Contents and storage

Contents	Cat. No.	Amount	Storage	Shelf life ^[1]
B-27™ Supplement Xeno-Free minus Vitamin A (50X)	A3353501	10 mL	-20°C to -5°C; Protect from light.	12 months

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

Culture conditions

Media: Complete CTS™ Neurobasal™ -A Medium or CTS™ Neurobasal™ Medium

Culture type: Suspension or adherent

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 5% CO₂. Ensure that proper gas exchange and minimize exposure of cultures to light.

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

- **Suspension culture:** Use pretreated “ultra low attachment” ULA cultureware (Nunc™ Sphera™ Dishes); alternatively, treat cultureware with anti-adhesive solution (TPP, poly (2-hydroxyethyl methacrylate).
- **Monolayer culture:**
 - a. Incubate tissue cultureware with fibronectin (1 µg/mL in CTS™ DPBS without calcium chloride, without magnesium chloride) for 1–24 hours at 37°C or (2% in CELLStart™ Substrate) for 1–2 hours at 37°C.
 - b. Aspirate solution and seed cell directly. No washing step is required.

Note: If precoating the day before use, store the coated culture vessels at 4°C sealed with Parafilm™ film to avoid drying.

Procedural guidelines

- Do not subject B-27™ Supplement Xeno-Free minus Vitamin A to repeated freeze/thaw cycles. Store B-27™ Supplement Xeno-Free minus Vitamin A in a non-frost-free freezer at -20°C.
- B-27™ Supplement Xeno-Free minus Vitamin A does not contain retinyl acetate, a component of B-27™ Supplement Xeno-Free, which can substitute for retinoic acid to stimulate differentiation of neural precursor/stem cells (NSCs).
- B-27™ Supplement Xeno-Free minus Vitamin A includes a cocktail of antioxidants to reduce reactive oxygen damage.
- CTS™ Neurobasal™ -A Medium (1X) is recommended for the serum-free growth of NSCs; CTS™ Neurobasal™ Medium and CTS™ KnockOut™ DMEM/F12 may also be used.
- Aseptic supplementation with EGF Recombinant Human Protein and CTS™ Recombinant Human FGF basic (full length) in addition to B-27™ Supplement Xeno-Free minus 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% Human serum albumin or medium), aliquot in working volumes and store at -20°C.

Guidelines for use

- B-27™ Supplement Xeno-Free minus Vitamin A when used as a supplement to CTS™ Neurobasal™ Medium (pre-natal/ embryonic cells), CTS™ Neurobasal™ -A Medium (post-natal/adult) or CTS™ KnockOut™ DMEM/F12 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 Xeno-Free minus 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.

Prepare complete medium

1. Aseptically add CTS™ GlutaMAX™ -I Supplement to 2–4 mM final concentration (10–20 mL/L) to CTS™ Neurobasal™ -A Medium before use.

2. Aseptically add 2% B-27™ Supplement Xeno-Free minus Vitamin A (20 mL/L) to the medium before use.

Note: Remaining B-27™ Supplement Xeno-Free minus 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 Xeno-Free minus Vitamin A more than twice.

Note: Once supplemented, the complete CTS™ 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.

Subculture cells

Subculture neurosphere suspension cultures

1. Collect and transfer neurospheres into a sterile centrifuge tube when they have reached ~100 µm in diameter.
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 smallbore 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.

Subculture monolayer cultures

1. Passage cells when the culture is 80–100% confluent.
2. Detach cells using CTS™ TrypLE™ Enzyme.
3. Transfer cells to a sterile centrifuge tube, centrifuge at 300 × g for 3 minutes, then discard the spend medium.
4. Resuspend the cell pellet in 1–2 mL pre-warmed complete medium.
5. Determine viable cell density using a Countess™ II Automated Cell Counter.
6. Seed the cells at a density of 5×10^4 viable cells/cm² in pre-warmed complete medium.

Differentiate NSCs

Neural stem cells cultured continuously according to the above instructions can be promoted to spontaneously differentiate into neurons by removing the growth factors.

Replace complete media with CTS™ Neurobasal™ Medium supplemented with 2% B-27™ Supplement Xeno-Free and 2 mM CTS™ GlutaMAX™ -I Supplement, 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

Item	Source
CTS™ Neurobasal™ Medium	A13712
CTS™ Neurobasal™-A Medium	A1371001
Nunc™ Sphera™ Dishes	174932
B-27™ Supplement Xeno-Free (50X)	A14867
CTS™ GlutaMAX™-I Supplement	A1286001
EGF Recombinant Human Protein	PHG0311
Fibronectin Human Protein, Plasma	33016
Insulin-Transferrin-Selenium (ITS -G) (100X)	41400
CTS™ N-2 Supplement	A13707
CELLStart™ Substrate	A1014201
Countess™ II Automated Cell Counter	AMQAX1000
Countess™ II FL Automated Cell Counter	AMQAF1000
Trypan Blue Stain	15250
CTS™ TrypLE™ Enzyme	A1285901
CTS™ KnockOut™ DMEM/F12	A1370801

Explanation of symbols

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

Limited product warranty

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Life Technologies Corporation | 3175 Staley Road | Grand Island, NY 14072

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