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

Catalog Number A3695201

Pub. No. MAN0017233 Rev. 2.0

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**.

Product description

Gibco™ B-27™ Supplement Xeno-Free minus Insulin is a B-27™ Supplement deficient in insulin. The absence of insulin makes this formulation ideal for the cultivation for the growth of hippocampal and other CNS neurons in applications where the presence of insulin causes interference or in the derivation of cardiomyocytes and mesodermal cell from stem cells. B-27™ Supplement Xeno-Free minus Insulin 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 Insulin (50X)	A3695201	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: Adherent

Temperature range: 36°C to 38°C

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

Culture vessels: Multi-well plates or T-flasks

Procedural guidelines

- Store B-27™ Supplement Xeno-Free minus Insulin in a non-frost-free freezer at –20°C.
- Thaw B-27™ Supplement Xeno-Free minus Insulin (50X) overnight at 4°C.

  Note: You may aliquot the remaining B-27™ Supplement Xeno-Free minus Insulin (50X) into working volumes and store at –20°C to –5°C.
- Do not freeze-thaw B-27™ Supplement Xeno-Free minus Insulin more than twice.
- B-27™ Supplement Xeno-Free minus Insulin includes a cocktail of antioxidants to reduce reactive oxygen damage.
- CTS™ Neurobasal™ Medium and CTS™ Neurobasal™-A Medium require supplementation with CTS™ GlutaMAX™-I Supplement (200 mM) and B-27™ Supplement Xeno-Free minus Insulin (50X) prior to use.

Guidelines for use

- Use B-27™ Supplement Xeno-Free minus Insulin to supplement CTS™ Neurobasal™ Medium for optimal viability and long-term survival of pre-natal and embryonic neuronal cells.
- Use B-27™ Supplement Xeno-Free minus Insulin to supplement CTS™ Neurobasal™-A Medium for optimal viability and long-term survival of post-natal and adult brain neuronal cells.
- B-27™ Supplement Xeno-Free minus Insulin when used as a supplement to RPMI 1640 Medium has been demonstrated to support differentiation of pluripotent stem cells into cardiomyocytes.
- Areas of application include: neural development, neural differentiation, diabetic neuropathy, and pluripotent stem cell differentiation.

Prepare complete medium

Prior to use, supplement CTS™ Neurobasal™-A Medium or CTS™ Neurobasal™-A Medium with CTS™ GlutaMAX™-I Supplement and B-27™ Supplement Xeno-Free minus Insulin.

1. Aseptically add CTS™ GlutaMAX™-I Supplement (200 mM) to 0.5 mM final concentration (2.5 mL/L).

2. Aseptically add 2% B-27™ Supplement Xeno-Free minus Insulin (50X) (20 mL/L).

3. Once supplemented, the complete CTS™ Neurobasal™-A Medium minus insulin is stable for up to one week when stored in the dark at 2°C to 8°C.

Guidelines to recover and culture cryopreserved neurons

- 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.
- Thaw one vial at a time. Transfer cryovial from liquid nitrogen storage to 37°C water bath minimizing handling time. Place a small amount of liquid nitrogen in an ice bucket to transport the vials from liquid nitrogen to the 37°C water bath.

Recover and culture cryopreserved neurons

1. Rinse a sterile 15-mL conical culture tube with complete medium minus insulin 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.

5. Collect the liquid to the bottom of the vial by gently tapping the vial on the hood's surface.

6. Use a pre-rinsed 1-mL pipette tip to very gently transfer the cells to the pre-rinsed 15-mL conical tube.

7. Rinse the cryovial with 1 mL of pre-warmed complete medium minus insulin 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.

8. Slowly (dropwise) add an additional 2 mL of pre-warmed complete medium minus insulin to the tube (for a total suspension volume of 4 mL).

9. Mix the suspension very gently with 1-mL pipette without creating any air bubbles.

10. 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.

11. Determine the viable cell density using a manual (i.e., hemocytometer) counting method.

    The viability of thawed cells should be >50%.

12. Plate ~1 × 10^5 cells (or desired cell density) per well in a poly-D-lysine and Laminin coated 48-well plate.

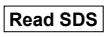
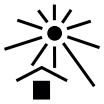
13. Dilute cell suspension to 500 µL per well by adding pre-warmed complete medium minus insulin.

14. 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 and maintain culture 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).

Related products

Item	Source
CTS™ Neurobasal™ Medium	A13712
CTS™ Neurobasal™-A Medium	A1371001
Insulin, human recombinant, zinc solution	12585014
CTS™ GlutaMAX™-I Supplement	A1286001
RPMI 1640 Medium (1X)	11875
Fibronectin Human Protein, Plasma	33016
rhLaminin-521	A29248
CTS™ DPBS without calcium chloride, without magnesium chloride	A1285601
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

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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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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