B-27™ Supplement Xeno-Free

Catalog Number A1486701

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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**.

Product description

Gibco™ B-27™ Supplement Xeno-Free is a complete, serum-free B-27™ Supplement formulated with only recombinant or humanized components. This B-27™ Supplement supports the low- or high-density growth and short- or long-term viability of hippocampal and other CNS neurons in applications requiring the absence of non-human origin components. B-27™ Supplement Xeno-Free is provided as a 50X liquid and can be used for neural differentiation of pluripotent stem cells (PSCs), proliferation of progenitor cells, and neuronal cell cultures.

Contents and storage

Contents	Cat. No.	Amount	Storage	Shelf life[1]
B-27™ Supplement Xeno-Free	A1486701	10 mL	–20°C to –5°C; Protect from light	12 months

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

Procedural guidelines

- Thaw frozen B-27™ Supplement Xeno-Free at 2-8°C overnight or at 37°C just until a small crystal remains in the tube. Do not overexpose the supplement to 37°C.

  Note: You may aliquot the remaining B-27™ Supplement Xeno-Free into working volumes and store at –20°C to –5°C.
- Do not freeze-thaw B-27™ Supplement Xeno-Free more than twice.

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.

Culture primary neurons

1. Prepare matrix with poly-L-lysine or poly-D-lysine according to manufacturer's recommendations. Briefly, prepare a working solution of the matrix at 10 µg/mL in water to cover the culture dish, and incubate it at room temperature for at least 1 hour.

2. Prepare 100 mL of complete medium by aseptically mixing the components listed below. Complete medium is stable for up to 2 weeks when stored in the dark at 4°C.

Component	Concentration	Amount
CTS™ Neurobasal™ Medium	1X	97 mL
CTS™ GlutaMAX™-I Supplement	2 mM	1 mL
Ascorbic acid[1]	200 µM	100 µL
B-27™ Supplement Xeno-Free	2%	2 mL

[1] Prepare 200 mM of ascorbic acid (Sigma, Cat. no. A8960) in water, aliquot, and freeze. Use the frozen aliquots within 3 months.

3. Allocate the appropriate amount of complete medium for the day and pre-warm to 37°C.

4. Rinse the coated dish with sterile water twice and add an appropriate volume of pre-warmed complete medium.

5. Plate the cells on the coated culture dish at a final seeding density of 2 × 10^4–5 ×10^4 cells/cm^2.

6. Replace the medium with fresh medium the next day, and change the medium every 2–3 days thereafter.

Differentiate neural stem cells to neurons

1. Prepare matrix with laminin according to manufacturer's recommendations. Briefly, prepare a working solution of laminin at 10 µg/mL in DPBS to cover the culture dish, and incubate it at 37°C for at least 1 hour.

2. Prepare complete medium according to the following table.

Component	Concentration	Amount
CTS™ Neurobasal™ Medium	1X	97 mL
CTS™ GlutaMAX™-I Supplement	2 mM	1 mL
Ascorbic acid[1]	200 µM	100 µL
B-27™ Supplement Xeno-Free	2%	2 mL

[1] Prepare 200 mM of ascorbic acid (Sigma, Cat. no. A8960) in water, aliquot, and freeze. Use the frozen aliquots within 3 months.

3. Allocate the appropriate amount of complete medium for the day and pre-warm to 37°C.

4. Aspirate the laminin solution from the culture dish and immediately add the appropriate amount of medium to prevent culture dish from drying.

5. Plate the cells on the coated culture dish at a final seeding density of 1 × 10^5 cells/cm^2.

6. Replace the medium with fresh medium the next day, and change one-half volume of the medium every 2–3 days thereafter.

Proliferate neural stem cells

1. Prepare matrix either with laminin (see above) or CELLStart™ Substrate according to manufacturer's recommendations. Briefly, prepare a working solution of CELLStart™ Substrate by diluting it 1:100 in DPBS with calcium and magnesium. Cover the culture dish with the dilute CELLStart™ Substrate and incubate it at 37°C for at least 1 hour.

2. Prepare 100 mL of complete proliferation medium by aseptically mixing the components listed below. Complete proliferation medium is stable for up to 2 weeks when stored in the dark at 4°C.

Component	Concentration	Amount
CTS™ KnockOut™ DMEM/F12	1X	96 mL
CTS™ GlutaMAX™-I Supplement	2 mM	1 mL
bFGF[1]	20 ng/mL	100 µL
EGF[1]	20 ng/mL	100 µL
CTS™ N-2 Supplement	1%	1 mL
B-27™ Supplement Xeno-Free	2%	2 mL
Ascorbic acid[2]	200 µM	100 µL

[1] Prepare 20 µg/mL of bFGF and EGF stock solution in 0.1% HSA containing DPBS with calcium and magnesium, aliquot, and freeze. Use the frozen aliquots within 6 months.

[2] Prepare 200 mM of ascorbic acid (Sigma, Cat. no. A8960) in water, aliquot, and freeze. Use the frozen aliquots within 3 months.

3. Allocate the appropriate amount of complete proliferation medium for the day and pre-warm to 37°C.

4. Aspirate the coating solution from the culture dish and immediately add the appropriate amount of proliferation medium to prevent the culture dish from drying.

5. Plate the cells on the coated culture dish at a final seeding density of 1 × 10^5 cells/cm^2.

6. Replace the medium with fresh complete proliferation medium the next day, and change the medium every other day thereafter.

7. Once the culture has reached to full confluency, passage the cells at a split ratio of 1:4 or at a seeding density of 1 × 10^5 cells/cm^2 as described below.

8. Prepare the dissociation enzyme by diluting the CTS™ TrypLE™ Enzyme to 0.5X in DPBS without calcium and magnesium.

9. Rinse the cells to be passaged with DPBS without calcium and magnesium.

10. Add the 0.5X CTS™ TrypLE™ Enzyme solution to the cells and incubate for 3 minutes at room temperature or at 37°C. If the culture is dense, increase the incubation time until the cells start to round up and separate from the culture dish.

11. Gently pipet the cells up and down to break the larger clumps into a single cell suspension.

12. Stop the dissociation reaction by adding complete proliferation medium at 4X the volume of 0.5X CTS™ TrypLE™ Enzyme solution. Disperse the medium by pipetting it over the cell layer surface several times.

13. Transfer the cells to a 15-mL tube and centrifuge at 300 × *g* for 4 minutes.

14. Resuspend the cells in complete proliferation medium to a final concentration of 1×10^4 cells/µL.

15. Plate the cells on a coated culture dish at a final seeding density of 1×10^5 cells/cm^2.

16. Replace the medium with fresh complete proliferation medium the next day, and change the medium every other day thereafter.

Cryopreserve neural stem cells

1. Prepare 2X freezing medium consisting of 20% DMSO and 80% complete proliferation medium. Keep the freezing medium on ice until use.

2. Harvest the cells.

3. Resuspend the cells in complete proliferation medium at a density of 2×10^6 cells/mL.

4. Add the same amount of 2X freezing medium as the complete proliferation medium in step 3 to the resuspended cells in a drop-wise manner (the final concentration of DMSO in 1X freezing medium is 10%, and the final cell concentration is 1×10^6 cells/mL).

5. Transfer 1 mL (1×10^6 cells) aliquots of the cell suspension into cryovials. Achieve cryopreservation overnight in a controlled-rate freezing apparatus following standard procedures (1°C decrease per minute).

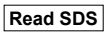
6. The next day, transfer the frozen vials to a liquid nitrogen tank (vapor phase) for long-term storage.

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
CTS™ Neurobasal™ Medium	A13712
CTS™ KnockOut™ DMEM/F12	A1370801
CTS™ GlutaMAX™-I Supplement	A1286001
EGF, Recombinant Human	PHG0311
CTS™ N-2 Supplement	A1370701
CELLStart™ Substrate	A10142
CTS™ TrypLE™ Enzyme	A12859

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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