gibco by life technologies™

B-27® Supplement XenoFree CTS™

Description

B-27® Supplement XenoFree CTS™ is a complete B-27® serum-free 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 XenoFree CTS™ 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. A broad array of CTS™ products are available for use in cell therapy research applications. CTS™ products deliver reduced burden in qualifying reagents when transitioning from research bench to the clinic with specific product use statements, full documentation traceability, and convenient access to our Drug Master File (DMF).

Product	Catalog no.	Amount	Storage	Shelf life*
B-27® Supplement XenoFree CTS™**	A14867-01	10 mL	Store at –5°C to –20°C. Protect from light.	12 months

* Shelf life duration is determined from Date of Manufacture.

** Do not subject B-27® XF Supplement to repeated freeze/thaw cycles. We recommend storing B-27® XF Supplement in a **non-frost-free** freezer at –20°C.

Product use

For Research Use or Manufacturing of Cell, Gene, or Tissue-Based Products. CAUTION: Not intended for direct administration into humans or animals.

Important information

- Thaw frozen B-27® XF Supplement at 2°C to 8°C overnight or at 37°C just until a small crystal remains in the tube. **Do not overexpose the supplement to 37°C.**
- Upon thawing, working aliquots of the supplement can be prepared and frozen for further use. Repeated freezing and thawing will cause the supplement to deteriorate.

Safety information

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

Caution: Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV, and HB_sAg. Handle in accordance with established bio-safety practices.

Culturing 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
Neurobasal® Medium CTS™	1X	97 mL
GlutaMAX™-I CTS™	2 mM	1 mL
Ascorbic acid*	200 µM	100 µL
B-27® Supplement XF CTS™	2%	2 mL

***** 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.

Differentiating 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 consisting of Neurobasal® medium supplemented with GlutaMAX™-I, ascorbic acid, and B-27® XF Supplement as described in **Culturing primary neurons**.
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.

Proliferating neural stem cells

1. Prepare matrix either with laminin (see above) or CELLstart™ CTS™ substrate according to manufacturer's recommendations. Briefly, prepare a working solution of CELLstart™ CTS™ substrate by diluting it 1:100 in DPBS with calcium and magnesium. Cover the culture dish with the dilute CELLstart™ CTS™ solution 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
KnockOut™ DMEM/F-12 CTS™	1X	96 mL
GlutaMAX™-I CTS™	2 mM	1 mL
bFGF CTS™*	20 ng/mL	100 µL
EGF*	20 ng/mL	100 µL
N-2 Supplement CTS™	1%	1 mL
B-27® Supplement XF CTS™	2%	2 mL
Ascorbic acid	200 µM	100 µL

* 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.

****** 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 TrypLE™ Select 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 TrypLE™ Select 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 TrypLE™ Select 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.

Cryopreserving 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 as described in **Proliferating neural stem 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

Product	Cat. no.
Neurobasal® Medium CTS™	A13712
KnockOut™ DMEM/F-12 CTS™	A13708
GlutaMAX™-I CTS™	A12860
bFGF, Recombinant Human CTS™	CTP0261
EGF, Recombinant Human	PHG0311
N-2 Supplement CTS™	A13707
CELLstart™ CTS™	A10142
TrypLE™ Select CTS™	A12859

Explanation of symbols and warnings

The symbols present on the product label are explained below:

				
Caution, consult accompanying documents	Temp. limit	Keep away from light	Use by	See website
LOT	REF		STERILE A	
Batch code	Catalog number	Manufacturer	Sterilized using aseptic technique	

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Important licensing information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit **www.lifetechnologies.com/support**
For further assistance, email **techsupport@lifetech.com**