CultureCEPT™ Supplement (1000X)

Catalog Numbers A56799, A56800

Pub. No. MAN0029749 Rev. B

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. SDSs are available from thermofisher.com/support.

Product description

Gibco™ CultureCEPT™ Supplement (1000X) is a chemically defined cell culture supplement that helps reduce cellular stress and improves viability of stem cells, differentiated cells, and primary cells during a wide variety of handling and processing steps. Comprising several small molecules, including chroman I, emricasan, polyamines, and trans-ISRIB, CultureCEPT™ Supplement delivers cytoprotective effects that improve performance in pluripotent stem cell (PSC) applications including recovery from cryopreservation, passaging, electroporation, and clonal expansion, as well as primary and PSC-derived neuron recovery from cryopreservation.

Contents and storage

Contents	Cat. No.	Amount	Storage	Shelf life[1]
CultureCEPT™ Supplement (1000X)	A56799	0.5 mL	-25°C to -5°C. Protect from light.	18 months
	A56800	0.1 mL		

[1] Shelf-Life duration is determined from Date of Manufacture. See Certificate of Analysis for expiry date.

Procedural guidelines

- Thaw CultureCEPT™ Supplement at room temperature.
- Divide thawed CultureCEPT™ Supplement into usage-size aliquots and store at -25°C to -5°C.
- Vortex CultureCEPT™ Supplement for 5 seconds before diluting.
- Dilute CultureCEPT™ Supplement 1:1000 into medium, and gently mix by inverting the tube/bottle at least 8 times.
- Include CultureCEPT™ Supplement in medium during desired cell culture step and/or for 24 hours following the step.

Recover cryopreserved PSCs

1. Coat the culture vessels with the appropriate substrate on which to culture your PSCs.
2. Quickly thaw cryopreserved PSCs in a 37°C waterbath until only a small ice crystal remains.
3. Gently pipet the thawed cells up and down to create a cell suspension and transfer the cell suspension into a 50-mL conical tube.
4. Dilute the cell suspension with 3 mL of medium, adding it dropwise while gently rocking the tube back and forth to avoid osmotic shock to the cells.
5. Centrifuge the cell suspension at 200 × *g* for 4 minutes.
6. Aspirate the medium, being careful not to disturb the cell pellet.
7. Gently resuspend the cells in medium supplemented with CultureCEPT™ Supplement at a 1X final concentration (i.e., 10 µL of CultureCEPT™ Supplement in 10 mL of medium).

   Note: Do not include additional ROCK inhibitors in the medium.
8. Transfer the cell suspension to the culture vessel pre-coated with the appropriate substrate. Move the vessel in several quick back-and-forth and side-to-side motions to disperse the cells across its surface.
9. Incubate the cells for 18–24 hours in the recommended cell culture environment.
10. Following incubation, aspirate the medium supplemented with CultureCEPT™ Supplement and replace it with medium that does not contain CultureCEPT™ Supplement.

Recover single-cell passaged PSCs

1. Coat the culture vessels with the appropriate substrate on which to culture your PSCs.
2. Pre-warm the dissociation reagent in a 37°C waterbath.
3. Aspirate the spent medium from the culture vessel.
4. Rinse the vessel once with the appropriate volume of Dulbecco's phosphate-buffered saline (DPBS) without calcium or magnesium.
5. Add the appropriate volume of pre-warmed dissociation reagent to the vessel.
6. Incubate the vessel at 37°C, 5% CO_2 for 5 minutes, continually observing the wells for cell detachment.
7. Gently pipet the cells up and down 5–10 times to generate single cell suspension.
8. Transfer the cell suspension to a conical tube containing the appropriate volume of culture medium to dilute the dissociation reagent.
9. Centrifuge the cells at 200 × *g* for 4 minutes.
10. Discard the supernatant. Flick the tube 3–5 times to loosen the pellet, and then resuspend the cells by pipetting them up and down 5–10 times in the recommended volume of medium supplemented with CultureCEPT™ Supplement at a 1X final concentration.
11. Determine viable cell density and percent viability using a Countess™ II Automated Cell Counter or a similar automated or manual method.
12. Adjust the concentration of the cell suspension using medium containing CultureCEPT™ Supplement to achieve the cell seeding density recommended for your culture vessel. The intended seeding density should account for the greater number of viable cells achieved with CultureCEPT™ Supplement.

    Note: Do not include additional ROCK inhibitors in the medium.
13. Transfer the cell suspension to the culture vessel pre-coated with the appropriate substrate. Move the vessel in several quick back-and-forth and side-to-side motions to disperse the cells across its surface.
14. Incubate the cells for 18–24 hours in the recommended cell culture environment.
15. Following incubation, aspirate the medium supplemented with CultureCEPT™ Supplement and replace it with medium that does not contain CultureCEPT™ Supplement, until the next passage.

Recover cryopreserved neurons

1. Quickly thaw cryopreserved neurons in a 37°C waterbath until only a small ice crystal remains.
2. Recover the cells according to the recommended protocol for your cell type. Perform cell count.
3. Prepare the cells for seeding by diluting the cell suspension in medium containing CultureCEPT™ Supplement at a 1X final concentration (i.e., 10 µL of CultureCEPT™ Supplement in 10 mL of medium).

   Note: Do not include additional ROCK inhibitors in the medium.
4. Transfer the cell suspension to the appropriate culture vessel and incubate for 18–24 hours in the recommended cell culture environment.
5. Following incubation, add an equal volume of medium that does not contain CultureCEPT™ Supplement. Perform a half-medium exchange using medium without CultureCEPT™ Supplement every 2–4 days (as needed) for the remainder of the culture.

Related products

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

Item	Source
Reagents	
Essential 8™ Medium	A1517001
Essential 8™ Flex Medium	A2858501
StemFlex™ Medium	A3349401
StemScale™ PSC Suspension Medium	A4965001
B-27™ Plus Neuronal Culture System	A3653401
StemPro™ Accutase™ Cell Dissociation Reagent	A1110501
DPBS, no calcium, no magnesium	14190144
Primary neurons	
Primary Mouse Cortical Neurons: 1 × 10^6 viable cells/vial, 4 × 10^6 viable cells/vial	A15585, A15586
Primary Mouse Hippocampus Neurons: 1 × 10^6 viable cells/vial	A15587
Instruments and consumables	
Countess™ II Automated Cell Counter	AMQAX1000

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.

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

Revision history: Pub. No. MAN0029749 B

Revision	Date	Description
B	7 May 2025	Updated product shelf life to 18 months. Added note to see Certificate of Analysis for expiry date to the Shelf life footnote.
A.0	18 August 2023	New document for CultureCEPT™ Supplement (1000X).