CTS™ PSC Cryopreservation Kit

Catalog Number A4239301

Pub. No. MAN0018276 **Rev.** 1.0

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™ CTS™ PSC Cryopreservation Kit contains a xeno-free cryopreservation medium, CTS™ PSC Cryomedium, and a chemically defined recovery supplement, CTS™ RevitaCell™ Supplement. When used in combination, the CTS™ PSC Cryopreservation Kit reagents allow for maximum post-thaw viability and recovery of cryopreserved pluripotent stem cells (PSCs). Using this kit, PSCs may be cryopreserved and recovered as clumps or as single cells, affording maximum flexibility in experimental workflow.

Contents and storage

Table 1 CTS™ PSC Cryopreservation Kit (Cat. No. A4239301)

Contents[1]	Cat. No.	Amount	Storage	Shelf Life[2]
CTS™ PSC Cryomedium	A4238801	50 mL	Store at –20°C to –5°C. Protect from light	12 months
CTS™ RevitaCell™ Supplement (100X)	A4238401	5 mL		

[1] CTS™ PSC Cryomedium and CTS™ RevitaCell™ Supplement (100X) are available separately.
[2] Shelf Life duration is determined from Date of Manufacture.

Procedural guidelines

- Once thawed, store CTS™ PSC Cryomedium at 2°C to 8°C until further use. Material has been shown to be stable up to 6 months from date of manufacture using this storage condition.
- Divide thawed CTS™ RevitaCell™ Supplement (100X) into usage-size aliquots and store in a non-frost-free freezer at –20°C to –5°C.

Cryopreserve PSCs

1. Thaw and pre-chill CTS™ PSC Cryomedium at 2°C to 8°C.
2. Harvest PSCs according to standard single or clumped cell passaging protocols.

   Note: Recommended passaging reagents for use with CTS™ Essential 8™ Medium include EDTA (CTS™ Versene Solution) for clumped cell passaging or CTS™ TrypLE™ Select Enzyme for single-cell passaging.
3. Centrifuge the cell suspension at 200 × *g* for 4 minutes.
4. Aspirate the medium, being careful not to disturb the cell pellet.
5. Add CTS™ PSC Cryomedium (chilled to 2°C to 8°C) dropwise to the cells while gently rocking the tube back and forth, followed by gentle resuspension of cell pellet.

   In general, from a 100-mm dish, 8–12 vials containing 1×10^6 viable cells/mL can be generated.
6. Dispense aliquots of the suspension into cryogenic vials according to manufacturer's specifications (i.e., 1.5 mL in a 2-mL cryovial).

   Mix the cell suspension in CTS™ PSC Cryomedium frequently to maintain a homogenous suspension. If utilizing clumped passaging methods at cell harvest, then mix cell suspension by gentle inversion to prevent breaking cells into smaller clumps.
7. Cryopreserve cells in an automated or manual controlled rate freezing apparatus following standard procedures (approximately 1°C decrease per minute).
8. Transfer frozen cell vials to liquid nitrogen (vapor phase). We recommend storage at –200°C to –125°C.

Recover cryopreserved PSCs

1. Coat a culture vessel with the appropriate substrate on which to culture your PSCs.
2. Quick-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 to a 50-mL conical tube.
4. Dilute the cell suspension with 3 mL of growth medium, adding it dropwise while gently rocking the tube back and forth to avoid osmotic shock to the cells.
5. Centrifuge cell suspension at 200 × *g* for 4 minutes.
6. Aspirate the medium.

   Be careful not to disturb the cell pellet.
7. Gently resuspend the cells in growth medium supplemented with CTS™ RevitaCell™ Supplement at a 1X final concentration (i.e., 100 µL of CTS™ RevitaCell™ Supplement in 10 mL of growth medium).

   Note: Do not add any additional ROCK inhibitors to the growth medium.
8. Transfer the cell suspension to an appropriate pre-coated culture vessel.

   Note: See "Recommended plating conditions".
9. Move the vessel in several quick back-and-forth and side-to-side motions to disperse the cells across its surface.
10. Incubate the cells for 18–24 hours in the recommended cell culture environment.
11. Following incubation, aspirate the growth medium supplemented with CTS™ RevitaCell™ Supplement and replace it with unsupplemented growth medium (i.e., without the addition of CTS™ RevitaCell™ Supplement) for the remainder of the culture.

Recommended plating conditions

Table 2 Recommended cell seeding densities and volumes of medium for plating (per well or per dish)

Culture vessel (surface area)	Number of viable cells added[1]		CTS™ Essential 8™ Medium + 1X CTS™ RevitaCell™ Supplement[2]
	20,000 cells/cm^2	40,000 cells/cm^2	
6-well (10 cm^2)	200,000	400,000	2 mL
12-well (4 cm^2)	80,000	160,000	1 mL
24-well (2 cm^2)	40,000	80,000	0.5 mL
35-mm (10 cm^2)	200,000	400,000	2 mL
60-mm (20 cm^2)	400,000	800,000	4 mL
100-mm (60 cm^2)	1,200,000	2,400,000	12 mL

[1] Time to confluency is 4–5 days for a seeding density of 20,000 cells/cm^2 and 3–4 days for a seeding density of 40,000 cells/cm^2
[2] For resuspension

Related products

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

Item	Source
CTS™ Essential 8™ Medium	A2656101
CTS™ Vitronectin (VTN-N) Recombinant Human Protein, Truncated	A27940
CTS™ Versene Solution	A42391
CTS™ TrypLE™ Select Enzyme	A12859
CTS™ DPBS without calcium chloride, without magnesium chloride	A12856
CTS™ PSC Cryomedium	A42388
CTS™ RevitaCell™ Supplement (100X)	A42384

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