CTS™ RevitaCell™ Supplement (100X)

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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™ CTS™ RevitaCell™ Supplement (100X) is a chemically defined recovery supplement containing a specific ROCK inhibitor coupled with molecules that have antioxidant and free radical scavenger properties. Use CTS™ RevitaCell™ Supplement to achieve efficient and optimal post-thaw recovery of cryopreserved pluripotent stem cells (PSCs). CTS™ RevitaCell™ Supplement can also be used for the recovery of PSCs from single-cell passaging procedures using dissociation reagents such as CTS™ TrypLE™ Select Enzyme.

Contents and storage

Contents	Amount	Storage	Shelf Life[1]
CTS™ RevitaCell™ Supplement (100X)	5 mL	–20°C to –5°C. Protect from light	12 months

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

Procedural guidelines

Divide thawed CTS™ RevitaCell™ Supplement (100X) into usage-size aliquots and store in a non-frost-free freezer at –20°C to –5°C.

Recover cryopreserved PSCs

1. Coat a culture vessel 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 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 the cell suspension at 200 × *g* for 4 minutes.
6. Aspirate the medium.

   Note: 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 reagent volumes and seeding densities".
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.

Recover single-cell passaged PSCs in CTS™ Essential 8™ Medium

1. Coat the culture vessels with the appropriate substrate on which to culture your PSCs.

   The recommended substrate is CTS™ Vitronectin (VTN-N) Recombinant Human Protein, Truncated (Cat. No. A27940).
2. Aspirate the spent medium from the culture vessel.
3. Rinse the vessel once with recommended volume of CTS™ DPBS without calcium chloride, without magnesium chloride (see Table 1).

4. Add recommended volume of CTS™ TrypLE™ Select Enzyme to the vessel (see Table 1).

5. Incubate the vessel at 37°C, 5% CO_2 for 5 minutes, continually observing the wells for cell detachment.

   Note: Avoid extended incubation of cells with dissociation reagents to minimize damage to cells.

6. Gently pipet the cells up and down 5–10 times to generate single cell suspension.

7. Transfer the cell suspension to a conical tube containing the recommended volume of CTS™ Essential 8™ Medium to dilute the dissociation reagent (see Table 1).

8. Centrifuge the cells at 200 × *g* for 4 minutes.

9. Discard the supernatant, flick the tube 3–5 times to loosen the pellet, and resuspend the cells by pipetting them up and down 5–10 times in the recommended volume of CTS™ Essential 8™ Medium supplemented with CTS™ RevitaCell™ Supplement at a 1X final concentration (see Table 1).

10. Determine viable cell density and percent viability using a Countess™ II Automated Cell Counter or a similar automated or manual method.

11. Adjust the concentration of the cell suspension using CTS™ Essential 8™ Medium supplemented with CTS™ RevitaCell™ Supplement at a 1X final concentration to achieve the cell seeding density recommended for your culture vessel (see Table 2).

    Note: Do not add any additional ROCK inhibitors to the growth medium.

12. Transfer the cell suspension to the pre-coated culture vessel.

    Move the vessel in several quick back-and-forth and side-to-side motions to disperse the cells across its surface.

13. Incubate the cells for 18–24 hours in the recommended cell culture environment.

14. Following incubation, aspirate the CTS™ Essential 8™ Medium supplemented with CTS™ RevitaCell™ Supplement and replace it with unsupplemented CTS™ Essential 8™ Medium (i.e., without the addition of CTS™ RevitaCell™ Supplement) for the remainder of the culture.

Recommended reagent volumes and seeding densities

Table 1 Reagent volumes (per well or per dish)

Culture vessel (surface area)	CTS™ DPBS for wash	CTS™ TrypLE™ Select Enzyme	CTS™ Essential 8™ Medium[1]	CTS™ Essential 8™ Medium + 1X CTS™ RevitaCell™ Supplement[2]
6-well (10 cm^2)	2 mL	1 mL	3 mL	2 mL
12-well (4 cm^2)	1 mL	0.4 mL	1.2 mL	1 mL
24-well (2 cm^2)	0.5 mL	0. 2 mL	0.6 mL	0.5 mL
35-mm (10 cm^2)	2 mL	1 mL	3 mL	2 mL
60-mm (20 cm^2)	4 mL	2 mL	6 mL	4 mL
100-mm (60 cm^2)	12 mL	6 mL	18 mL	12 mL

[1] For dilution
[2] For resuspension

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]
	12,500 cells/cm^2	25,000 cells/cm^2	
6-well (10 cm^2)	125,000	250,000	2 mL
12-well (4 cm^2)	50,000	100,000	1 mL
24-well (2 cm^2)	25,000	50,000	0.5 mL
35-mm (10 cm^2)	125,000	250,000	2 mL
60-mm (20 cm^2)	250,000	500,000	4 mL
100-mm (60 cm^2)	750,000	1,500,000	12 mL

[1] Time to confluency is 4–5 days for a seeding density of 12,500 cells/cm^2 and 3–4 days for a seeding density of 25,000 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™ PSC Cryopreservation Kit	A42393

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Life Technologies Corporation | 3175 Staley Road | Grand Island, NY 14072
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