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RevitaCell™ Supplement (100X)

Description

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 RevitaCell™ Supplement to achieve efficient and optimal post-thaw recovery of cryopreserved pluripotent stem cells (PSCs) as well as primary cells, including neurons, human epidermal keratinocytes (HEKs), and human corneal epithelial cells (HCECs). RevitaCell™ Supplement can also be used for the recovery of PSCs from single-cell passaging procedures using dissociation reagents such as TrypLE™ Select, TrypLE™ Express, and StemPro® Accutase®.

Product*	Catalog no.	Amount	Storage	Shelf life*
RevitaCell™ Supplement (100X)	A2644501	5 mL	–20°C to –5°C; Protect from Light	12 months

* Shelf Life duration is determined from Date of Manufacture.

Product use

For Research Use Only. Not for use in diagnostic procedures.

Safety information

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

Important information

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

Recovery of cryopreserved PSCs

1. Coat the culture vessels with the appropriate substrate on which to culture your PSCs. Recommended Substrates include Vitronectin (VTN-N) Recombinant Human Protein (Cat. no. A14700) and Geltrex® LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix (Cat. no. A14133).
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 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, being careful not to disturb the cell pellet.
7. Gently resuspend the cells in growth medium supplemented with RevitaCell™ Supplement at a 1X final concentration (i.e., 100 µL of 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 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 growth medium supplemented with RevitaCell™ Supplement and replace it with unsupplemented growth medium (i.e., without the addition of RevitaCell™ Supplement) for the remainder of the culture.

Recovery of cryopreserved primary cells

1. Quickly thaw cryopreserved primary cells 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 RevitaCell™ Supplement at a 1X final concentration (i.e., 100 µL of RevitaCell™ Supplement in 10 mL of growth medium).

   Note: Do not add any additional ROCK inhibitors to the growth 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, aspirate the growth medium supplemented with RevitaCell™ Supplement and replace it with unsupplemented growth medium (i.e., without the addition of RevitaCell™ Supplement) for the remainder of the culture.

Recovery of single-cell passaged PSCs in Essential 8® Medium

1. Coat the culture vessels with the appropriate substrate on which to culture your PSCs. Recommended Substrates include Vitronectin (VTN-N) Recombinant Human Protein (Cat. no. A14700) and Geltrex® LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix (Cat. no. A14133).
2. Pre-warm the required volume of TrypLE™ Select, TrypLE™ Express, or StemPro® Accutase® dissociation reagent in a 37ºC waterbath.
3. Aspirate the spent medium from the culture vessel.
4. Rinse the vessel once with recommended volume of Dulbecco's Phosphate Buffered Saline (DPBS) without calcium or magnesium (see Table 1).
5. Add recommended volume of prewarmed TrypLE™ Select, TrypLE™ Express, or StemPro® Accutase® dissociation reagent to the vessel (see Table 1).
6. Incubate the vessel at 37 ºC, 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.
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 recommended volume of Essential 8™ Medium to dilute the dissociation reagent (see Table 1).
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 Essential 8® Medium supplemented with RevitaCell™ Supplement at a 1X final concentration (see Table 1).
11. Determine viable cell density and percent viability using a Countess® Automated Cell Counter or a similar automated or manual method.
12. Adjust the concentration of the cell suspension using Essential 8® Medium supplemented with 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.
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 Essential 8® Medium supplemented with RevitaCell™ Supplement and replace it with unsupplemented Essential 8® Medium (i.e., without the addition of RevitaCell™ Supplement) for the remainder of the culture.

Table 1 Reagent volumes (per well or per dish)

Culture vessel (surface area)	DPBS for wash	Dissociation reagent*	Essential 8® Medium**	Essential 8® Medium + 1X RevitaCell™ Supplement***
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

* TrypLE™ Express, TrypLE™ Select, or StemPro® Accutase® reagent
** For dilution *** 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*		Essential 8® Medium + 1X RevitaCell™ Supplement**
	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

* 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 12,500 cells/cm^2
** For resuspension

Related Products

Product	Cat. no.
Essential 8® Medium	A15170
Vitronectin, truncated human recombinant (VTN-N)	A14700
Geltrex® LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix	A14133
UltraPure™ 0.5 M EDTA, pH 8.0	15575
StemPro® Accutase® Cell Dissociation Reagent	A11105
TrypLE™ Express, no phenol red	12604
TrypLE™ Select, no phenol red	12563
DPBS, no calcium, no magnesium	14190
Primary Rat Cortex Neurons	A10840
Neurobasal® Medium 1X, liquid	21103
B-27® Supplement 50X, liquid	17504
Human Epidermal Keratinocytes, neonatal (HEKn)	C0015C
Human Epidermal Keratinocytes, adult (HEKa)	C0055C
Human Corneal Epithelial Cells (HCEC)	C0185C
EpiLife® Medium, with 60 µM calcium	MEPI500CA
Human Keratinocyte Growth Supplement (HKGS) 100X	S0015
Human Corneal Growth Supplement (HCGS) 100X	S0095

Explanation of Symbols and Warnings

				
Caution, consult accompanying documents	Temperature Limitation	Keep away from light	Use By:	Consult instructions for use
				
Batch Code	Catalog number	Manufacturer	Sterilized using aseptic processing techniques	Read Safety Data Sheet

Limited product warranty

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