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PSC Cryopreservation Kit

Description

The PSC Cryopreservation Kit contains a xeno-free cryopreservation medium, PSC Cryomedium, and a chemically defined recovery supplement, RevitaCell™ Supplement. When used in combination, the 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. This kit can also be used for cryopreservation and recovery of peripheral blood mononuclear cells (PBMCs) to improve post-thaw cell viability and recovery.

Kit Name/Components*	Cat. no./Part no.	Amount	Storage	Shelf life**
PSC Cryopreservation Kit contains:	**A2644601**	**1 Kit**		
PSC Cryomedium	A2644401	50 mL	–20°C to –5°C; Protect from Light	12 months
RevitaCell™ Supplement (100X)	A2644501	5 mL	–20°C to –5°C; Protect from Light	12 months

* PSC Cryomedium (Cat. no. A2644401) and RevitaCell™ Supplement (Cat. no. A2644501) are also available separately.
** 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

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

Cryopreservation of PSCs

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

   Note: Recommended passaging reagents for use with Essential 8® Medium include EDTA (Versene) for clumped cell passaging, or TrypLE® Select or StemPro® Accutase® 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 PSC Cryomedium (chilled to 2ºC to 8ºC) dropwise to the cells while gently rocking the tube back and forth followed by gentle resuspension of cell pellet.

   Note: 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).

   Note: Mix the cell suspension in 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 1ºC decrease per minute).
8. Transfer frozen cell vials to liquid nitrogen (vapor phase); we recommend storage at –200°C to –125°C.

Recovery of cryopreserved PSCs

1. Quick-thaw cryopreserved PSCs in a 37°C waterbath until only a small ice crystal remains.
2. Gently pipet the thawed cells up and down to create a cell suspension and transfer to a 50-mL conical tube.
3. 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.
4. Centrifuge cell suspension at 200 × *g* for 4 minutes.
5. Aspirate the medium, being careful not to disturb the cell pellet.
6. 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.
7. Transfer the cell suspension to an appropriate growth vessel and incubate for 18–24 hours in the recommended culture environment.

   Note: See Table 1 for recommended cell seeding densities.
8. 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.

Cryopreservation of PBMCs

1. Isolate PBMCs using standard protocols for density gradient centrifugation and/or use of ACK Lysing Buffer.
2. Centrifuge the final cell suspension at 300 × *g* for 15 minutes.
3. Aspirate the medium, being careful not to disturb the cell pellet.
4. Add PSC Cryomedium (chilled to 2ºC to 8ºC) dropwise to the cells while gently rocking the tube back and forth followed by gentle resuspension of cell pellet.
5. Dispense aliquots of the suspension into cryogenic vials according to manufacturer's specifications (i.e., 1.5-mL in a 2-mL cryovial).

   Note: Mix the cell suspension frequently to maintain a homogenous suspension.
6. Cryopreserve cells in an automated or manual controlled rate freezing apparatus following standard procedures (approximately 1ºC decrease per minute).
7. Transfer frozen cell vials to liquid nitrogen (vapor phase); we recommend storage at –200°C to –125°C.

Recovery of cryopreserved PBMCs

1. Quick-thaw cryopreserved PBMCs in a 37°C waterbath until only a small ice crystal remains.
2. Gently pipet the thawed cells up and down to create a cell suspension and transfer to a 50-mL conical tube.
3. 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.
4. Centrifuge cell suspension at 400 × *g* for 10 minutes.
5. Aspirate the medium, being careful not to disturb the cell pellet.
6. Gently resuspend the cells in growth medium supplemented with RevitaCell™ Supplement.

   Note: Do not add any additional ROCK inhibitors to the growth medium.
7. Transfer the cell suspension to an appropriate growth vessel and incubate for 18–24 hours in the recommended culture environment.
8. Following incubation, refresh medium with unsupplemented growth medium (i.e., without the addition of RevitaCell™ Supplement) for the remainder of the culture.

Table 1 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**
	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

* 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
** 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™ Select, no phenol red	12563
DPBS, no calcium, no magnesium	14190
RPMI Medium 1640	A10491
ACK Lysing Buffer	A10492
HBSS	14185
RevitaCell™ Supplement (100X)	A2644501

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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