STEMdiff™ Cardiomyocyte Freezing Medium

Medium for cryopreserving hPSC-derived cardiomyocytes

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Catalog #05030 50 mL

Product Description

STEMdiff™ Cardiomyocyte Freezing Medium can be used to cryopreserve cardiomyocytes derived from human pluripotent stem cells (hPSCs) and maintained in STEMdiff™ Cardiomyocyte Maintenance Medium (Catalog #05020). STEMdiff™ Cardiomyocyte Dissociation Kit (Catalog #05025) is required for dissociating cardiomyocytes prior to freezing. After thawing using STEMdiff™ Cardiomyocyte Support Medium (Catalog #05027), the cardiomyocytes can be used in various downstream applications and analyses. This medium supports hPSC-derived cardiomyocytes generated using STEMdiff™ Cardiomyocyte Differentiation Kit (Catalog #05010).

Product Information

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Cardiomyocyte Freezing Medium*	05030	50 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.

*Please refer to the Safety Data Sheet (SDS) for hazard information. This product contains components dissolved in dimethyl sulfoxide (DMSO). DMSO is a strong solvent and skin penetrant, and can transport many substances through the skin. DMSO can also penetrate some protective glove materials including latex and silicone. Extra caution should be utilized when handling this product.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
STEMdiff™ Cardiomyocyte Dissociation Kit • STEMdiff™ Cardiomyocyte Dissociation Medium • STEMdiff™ Cardiomyocyte Support Medium (Catalog #05027)	05025
STEMdiff™ Cardiomyocyte Maintenance Kit • STEMdiff™ Cardiomyocyte Maintenance Basal Medium • STEMdiff™ Cardiomyocyte Maintenance Supplement (50X)	05020
Trypan Blue	07050

Directions for Use

Please read the entire protocol before proceeding. Use sterile techniques when performing the following protocols.

hPSC-derived cardiomyocytes can be harvested and cryopreserved as early as Day 15 of differentiation/maintenance.

A. CRYOPRESERVING hPSC-DERIVED CARDIOMYOCYTES

1. Dissociate/harvest hPSC-derived cardiomyocytes using STEMdiff™ Cardiomyocyte Dissociation Kit. Refer to the Product Information Sheet (PIS; Document #DX21497) for the complete protocol.
2. Centrifuge cell suspension at 300 x *g* for 5 minutes at room temperature (15 - 25°C).
3. Gently pour off the supernatant, taking care not to disrupt the cell pellet.
4. Gently resuspend the cells in STEMdiff™ Cardiomyocyte Freezing Medium to a final concentration of 5 x 10^5 cells/mL.
5. Transfer 1 mL of cell suspension into a labeled cryovial.
6. Freeze cell suspension using a standard slow rate-controlled cooling protocol that reduces temperatures at approximately -1°C/minute, followed by long-term storage at -135°C (liquid nitrogen) or colder. Long-term storage at -80°C is not recommended.

B. THAWING hPSC-DERIVED CARDIOMYOCYTES

Cryopreserved hPSC-derived cardiomyocytes should be thawed and plated onto Corning® Matrigel®-coated cultureware. For coating plates with Corning® Matrigel®, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #28315) or TeSR™-E8™ (Document #29267) available at www.stemcell.com or contact us to request a copy.

For storage, stability, and preparation instructions for STEMdiff™ Cardiomyocyte Support Medium and STEMdiff™ Cardiomyocyte Maintenance Medium, refer to the corresponding PIS's (Document #DX21497 and DX21496, respectively).

The following instructions are for a 12-well tissue culture plate. For other cultureware, adjust accordingly.

1. Coat a 12-well tissue culture plate with Corning® Matrigel® hESC-Qualified Matrix and bring to room temperature (15 - 25°C) for at least 1 hour prior to use.
2. Thaw STEMdiff™ Cardiomyocyte Support Medium and warm to room temperature (15 - 25°C).
3. Thaw hPSC-derived cardiomyocytes in a 37°C water bath by gently shaking the cryovial continuously until only a small frozen cell pellet remains.
4. Add 5 - 7 mL of STEMdiff™ Cardiomyocyte Support Medium to a 15 mL conical tube (e.g. Catalog #38009).
5. Using a 2 mL pipette, gently transfer the contents of the cryovial to the tube from step 4.
6. Centrifuge the cells at 300 x *g* for 5 minutes at room temperature (15 - 25°C).
7. Aspirate the supernatant and gently add 1 - 2 mL of STEMdiff™ Cardiomyocyte Support Medium to resuspend cells.
8. Perform a cell count using trypan blue and a hemocytometer.
9. Aspirate Corning® Matrigel® from the tissue culture plate prepared in step 1. Add 2 mL of STEMdiff™ Cardiomyocyte Support Medium per well.
10. Add cells at a density appropriate for downstream assays or other applications. Incubate at 37°C for 24 hours.
11. Remove medium and add 2 mL of STEMdiff™ Cardiomyocyte Maintenance Medium per well. Incubate at 37°C.
12. Every 2 days, perform a full medium change with 2 mL of STEMdiff™ Cardiomyocyte Maintenance Medium per well.

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