

STEMdiff™ Cardiomyocyte Dissociation Kit

For dissociation of hPSC-derived cardiomyocytes



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Catalog #05025

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Product Description

STEMdiff™ Cardiomyocyte Dissociation Kit includes STEMdiff™ Cardiomyocyte Dissociation Medium and STEMdiff™ Cardiomyocyte Support Medium. STEMdiff™ Cardiomyocyte Dissociation Medium can be used to harvest cardiomyocytes that have been differentiated from human pluripotent stem cells (hPSCs) using STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit (Catalog #05010) or STEMdiff™ Atrial Cardiomyocyte Kit (Catalog #100-0215) and maintained in STEMdiff™ Cardiomyocyte Maintenance Medium (Catalog #05020).

NOTE: To reduce stress on hPSC-derived cardiomyocytes during harvesting and replating, use of STEMdiff™ Cardiomyocyte Plating Supplement (Catalog #100-1120) in combination with STEMdiff™ Cardiomyocyte Support Medium is recommended. The addition of the STEMdiff™ Cardiomyocyte Plating Supplement increases the viability and plating efficiency of hPSC-derived cardiomyocytes.

Product Information

STEMdiff™ Cardiomyocyte Dissociation Medium and STEMdiff™ Cardiomyocyte Support Medium are sold as a complete kit (Catalog #05025). STEMdiff™ Cardiomyocyte Support Medium (Catalog #05027) and STEMdiff™ Cardiomyocyte Plating Supplement are also available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Cardiomyocyte Dissociation Kit (Catalog #05025)				
STEMdiff™ Cardiomyocyte Dissociation Medium	05026	50 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Cardiomyocyte Support Medium	05027	250 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
STEMdiff™ Cardiomyocyte Plating Supplement	100-1120	2.5 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.

Preparation of Media

A. STEMdiff™ CARDIOMYOCYTE DISSOCIATION MEDIUM

1. Thaw STEMdiff™ Cardiomyocyte Dissociation Medium at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.

NOTE: If not used immediately, store STEMdiff™ Cardiomyocyte Dissociation Medium at 2 - 8°C for up to 1 month. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the medium. Once aliquots are thawed, use immediately. Do not re-freeze.

B. STEMdiff™ CARDIOMYOCYTE PLATING MEDIUM

Use sterile technique to prepare complete STEMdiff™ Cardiomyocyte Plating Medium (STEMdiff™ Cardiomyocyte Support Medium + STEMdiff™ Cardiomyocyte Plating Supplement [100X]). The following example is for preparing 250 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw STEMdiff™ Cardiomyocyte Support Medium at room temperature or overnight at 2 - 8°C. Mix thoroughly.

NOTE: If not used immediately, store STEMdiff™ Cardiomyocyte Support Medium at 2 - 8°C for up to 1 month. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the medium. Once aliquots are thawed, use immediately. Do not re-freeze.

2. Thaw STEMdiff™ Cardiomyocyte Plating Supplement (100X) at room temperature. Mix thoroughly.

NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the Supplement. After thawing aliquots, use immediately. Do not re-freeze.

3. Add 2.5 mL of STEMdiff™ Cardiomyocyte Plating Supplement (100X) to 247.5 mL of STEMdiff™ Cardiomyocyte Support Medium. Mix thoroughly.

NOTE: If not used immediately, store complete STEMdiff™ Cardiomyocyte Plating Medium at 2 - 8°C for up to 2 weeks. Warm medium to room temperature before use.

C. STEMdiff™ CARDIOMYOCYTE MAINTENANCE MEDIUM

For storage, stability, and preparation instructions for STEMdiff™ Cardiomyocyte Maintenance Medium, refer to the applicable Product Information Sheet (PIS; Document #10000009775).

Directions for Use

Please read the entire protocol before proceeding. Use sterile technique when performing the following protocols. The following instructions are for dissociation of hPSC-derived cardiomyocytes that have been maintained in STEMdiff™ Cardiomyocyte Maintenance Medium in 1 well of a 12-well plate. Dissociation can be performed as early as Day 15 of differentiation/maintenance.

A. DISSOCIATING hPSC-DERIVED CARDIOMYOCYTES

1. Wash each well to be harvested with 2 x 1 mL of D-PBS (Without Ca⁺⁺ and Mg⁺⁺; Catalog #37350).
2. Gently remove the wash and add 1 mL/well of warm (37°C) STEMdiff™ Cardiomyocyte Dissociation Medium.
3. Incubate at 37°C and 5% CO₂ for 10 - 12 minutes.
4. Add 2 mL of Cardiomyocyte Plating Medium (see Preparation of Media, section B) per well. Dislodge cells by pipetting up and down 3 - 5 times using a 10 mL serological pipette.

Critical: Do not use a smaller-bore pipette tip at this step, as this may result in significant cell death.

5. Immediately transfer cells from one well to a tube containing 3 mL of STEMdiff™ Cardiomyocyte Plating Medium.
6. Centrifuge at 300 x g for 5 minutes. Remove and discard supernatant.
7. Gently resuspend cell pellet with 1 - 2 mL STEMdiff™ Cardiomyocyte Plating Medium.
8. Perform a cell count using an automated cell counter (e.g. NucleoCounter® NC-250™) or with Trypan Blue and a Hausser Scientific™ Bright-Line Hemocytometer (Catalog #100-1181).
9. Single-cell hPSC-derived cardiomyocytes are now ready for standard assays or replating. For more information, refer to the PIS for STEMdiff™ Cardiomyocyte Support Medium (Document #1000000170).

B. [OPTIONAL] REPLATING hPSC-DERIVED CARDIOMYOCYTES

The following instructions are for replating thawed or dissociated (section B) hPSC-derived cardiomyocytes onto a 12-well plate. For other cultureware, adjust volumes accordingly.

NOTE: For more information on thawing hPSC-derived cardiomyocytes, refer to the applicable PIS (Document #1000000171).

1. Coat a 12-well tissue culture plate with Corning® Matrigel® hESC-Qualified Matrix (Corning Catalog #354277) and bring to room temperature (15 - 25°C) for at least 1 hour prior to use.
NOTE: For coating plates with Matrigel®, refer to the Technical Manual for mTeSR™1, mTeSR™ Plus, or TeSR™-E8™, available at www.stemcell.com or contact us to request a copy.
2. Warm STEMdiff™ Cardiomyocyte Plating Medium and STEMdiff™ Cardiomyocyte Maintenance Medium (see Preparation of Media) to room temperature.
3. Aspirate Matrigel® from the coated 12-well tissue culture plate (prepared in step 1). Add 0.5 mL of STEMdiff™ Cardiomyocyte Plating Medium per well.
4. Add cells at a density appropriate for downstream assays or other applications. Incubate at 37°C for 24 hours.
5. Remove medium and add 2 mL of STEMdiff™ Cardiomyocyte Maintenance Medium per well. Incubate at 37°C.
6. The following day, warm STEMdiff™ Cardiomyocyte Maintenance Medium to room temperature.
7. Remove medium and add 2 mL of STEMdiff™ Cardiomyocyte Maintenance Medium per well. Incubate at 37°C and 5% CO₂.
8. Every 2 days, perform a full-medium change with 2 mL of STEMdiff™ Cardiomyocyte Maintenance Medium per well.

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