

STEMdiff™ Cardiomyocyte Differentiation Kit

For the Standardized Generation of Cardiomyocytes from hPSCs

The **STEMdiff™ Cardiomyocyte Differentiation Kit** consists of defined, serum-free basal media and supplements designed for the generation of cardiomyocytes from human pluripotent stem cells (hPSCs). Optimized for a standardized, 15-day differentiation protocol (Figure 1), this kit supports robust differentiation of hPSCs into cardiomyocytes (Figure 2) that can be identified by the expression of a key marker, cardiac troponin T (cTnT) (Figure 3). Contracting hPSC-derived cardiomyocytes can be seen as early as day 8. This kit is formulated for use in feeder-free conditions, optimized for the differentiation of hPSCs maintained in mTeSR™1 (Catalog #05850) or TeSR™-E8™ (Catalog #05940) and compatible with multiple human embryonic stem (hES) and induced pluripotent stem (hiPS) cell lines. After differentiation, the resulting cardiomyocytes may be used for additional downstream assays, such as disease modeling, drug discovery, and cardiotoxicity screening.

Complete Workflow

The STEMdiff™ Cardiomyocyte Differentiation Kit is part of the **STEMdiff™ Cardiomyocyte System**. The STEMdiff™ Cardiomyocyte System is composed of specialized media products that optimize the entire hPSC-derived cardiomyocyte research workflow, including differentiation and maintenance, dissociation and replating, and cryopreservation. Following hPSC differentiation to cardiomyocytes, the hPSC-derived cardiomyocytes can be maintained long-term in the STEMdiff™ Cardiomyocyte **Maintenance Kit**. The STEMdiff™ Cardiomyocyte **Dissociation Kit** allows for standardized harvesting of hPSC-derived cardiomyocytes that are ready for use in downstream applications, such as flow cytometry, immunocytochemistry, calcium imaging, electrophysiology, and cryopreservation. These cardiomyocytes can be cryopreserved using the STEMdiff™ Cardiomyocyte **Freezing Medium**. The STEMdiff™ Cardiomyocyte **Support Medium** maintains the viability of cardiomyocytes after thawing or during dissociation, harvesting or replating.

Advantages:

- COMPLETE.** Supports the entire hPSC-derived cardiomyocyte workflow.
- EASY-TO-USE.** Simple monolayer protocol produces cardiomyocytes in 15 days.
- HIGH YIELD.** One kit generates over 50 million cardiomyocytes expressing cTnT.
- STANDARDIZED.** Robust performance with minimal variability across multiple hPSC lines.

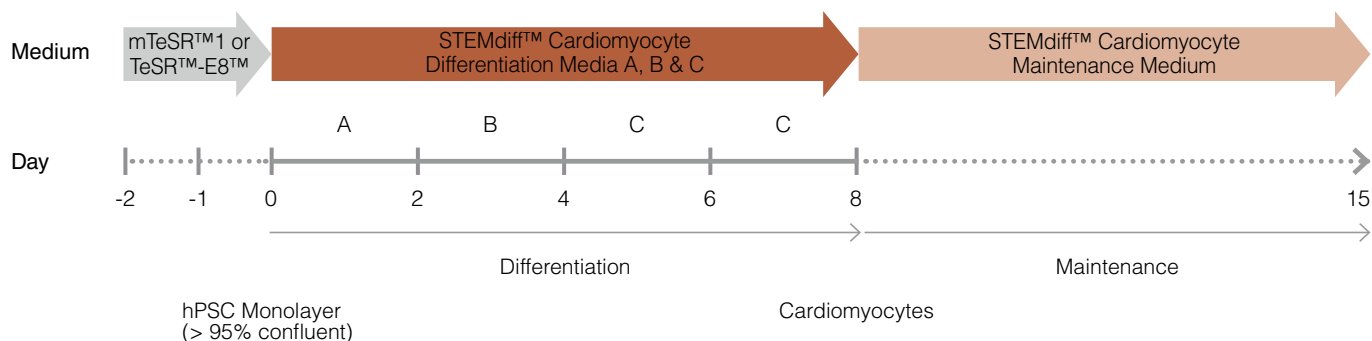


Figure 1. Cardiomyocyte Differentiation Protocol

Two days before the differentiation protocol, hPSC colonies are harvested and seeded as single cells at 350,000 cells/well in a 12-well format in mTeSR™1 or TeSR™-E8™. After one day (Day -1), the medium is replaced with fresh TeSR™ medium. The following day (Day 0), the TeSR™ medium is replaced with Medium A (STEMdiff™ Cardiomyocyte Differentiation Basal Medium containing Supplement A) to begin inducing the cells toward a cardiomyocyte fate. On day 2, a full medium change is performed with fresh Medium B (STEMdiff™ Cardiomyocyte Differentiation Basal Medium containing Supplement B). On days 4 and 6, full medium changes are performed with fresh Medium C (STEMdiff™ Cardiomyocyte Differentiation Basal Medium containing Supplement C). On day 8, medium is switched to STEMdiff™ Cardiomyocyte Maintenance Medium with full medium changes on days 10, 12 and 14, to promote further differentiation into cardiomyocyte cells. Small beating areas of cardiomyocytes can be seen as early as day 8, progressing to a full lawn of beating cardiomyocytes that can be harvested as early as day 15.



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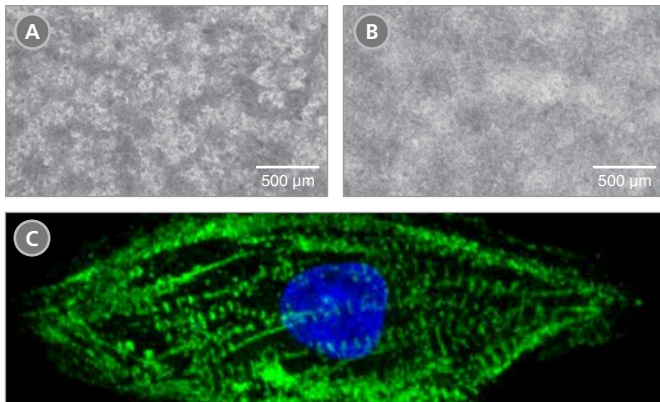


Figure 2. Morphology of hPSC-Derived Cardiomyocytes

Representative images of (A) hES (H9) cells and (B) hiPS (WLS-1C) cells on day 15 of differentiation to cardiomyocytes using the STEMdiff™ Cardiomyocyte Differentiation Kit. Differentiated cells exhibit typical cardiomyocyte morphology as an adherent, tightly packed web-like monolayer of beating cells. (C) Representative confocal microscopy image of a single hPSC-derived cardiomyocyte generated with the STEMdiff™ Cardiomyocyte Differentiation Kit and stained with cTnT (green) and DAPI (blue).

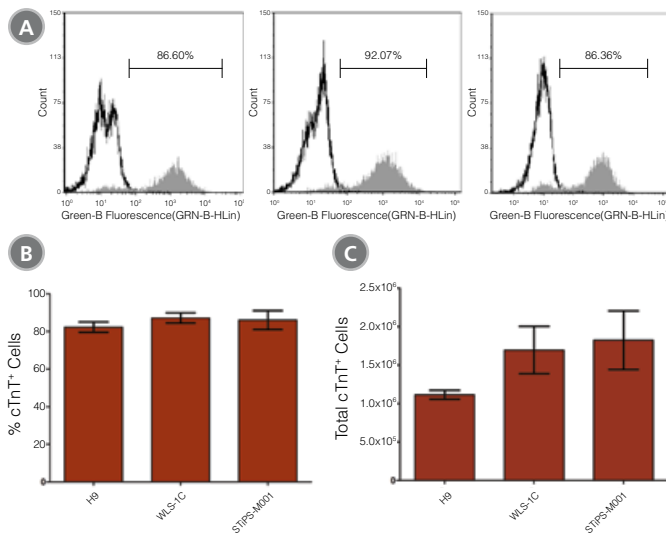


Figure 3. Efficient and Robust Generation of cTnT-Positive Cardiomyocytes

hES and hiPS cells were cultured for 15 days in single wells of 12-well plates using the STEMdiff™ Cardiomyocyte Differentiation Kit. At the end of the culture period, cells were harvested and analyzed by flow cytometry for expression of cardiac troponin T (cTnT). (A) Histogram analysis for cardiomyocyte cell marker cTnT for cultures of hES (H9) and hiPS (WLS-1C and STiPS-M001) cells. (Filled = sample; blank = secondary antibody only control) (B,C) Percentages and total numbers of cells expressing cTnT in cultures of hES or hiPS cells are shown. Data shown as mean ± SEM; n=3.

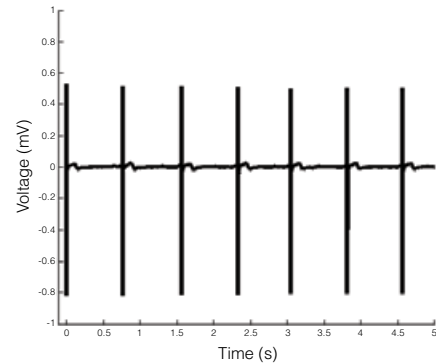


Figure 4. hPSC-Derived Cardiomyocytes Exhibit a Robust and Stable Excitability Profile

Microelectrode array (MEA) voltage recordings of cardiomyocytes (day 27) derived from human pluripotent stem cells generated and maintained with the STEMdiff™ Cardiomyocyte Differentiation and Maintenance Kits. The hPSC-derived cardiomyocytes have a characteristic electrical profile and stable beat rate. A large depolarization spike followed by a smaller repolarization deflection is observed.

PRODUCT	KIT COMPONENT	SIZE	CATALOG #
STEMdiff™ Cardiomyocyte Differentiation Kit		1 Kit	05010
	STEMdiff™ Cardiomyocyte Differentiation Basal Medium	380 mL	05011
	STEMdiff™ Cardiomyocyte Differentiation 10X Supplement A	10 mL	05012
	STEMdiff™ Cardiomyocyte Differentiation 10X Supplement B	10 mL	05013
	STEMdiff™ Cardiomyocyte Differentiation 10X Supplement C	10 mL	05014
	STEMdiff™ Cardiomyocyte Maintenance Basal Medium	490 mL	05015
	STEMdiff™ Cardiomyocyte Maintenance 50X Supplement	10 mL	05016
STEMdiff™ Cardiomyocyte Maintenance Kit		1 Kit	05020
	STEMdiff™ Cardiomyocyte Maintenance Basal Medium	490 mL	05015
	STEMdiff™ Cardiomyocyte Maintenance 50X Supplement	10 mL	05016
STEMdiff™ Cardiomyocyte Dissociation Kit		1 Kit	05025
	STEMdiff™ Cardiomyocyte Dissociation Medium	50 mL	05026
	STEMdiff™ Cardiomyocyte Support Medium	250 mL	05027
STEMdiff™ Cardiomyocyte Support Medium		250 mL	05027
STEMdiff™ Cardiomyocyte Freezing Medium		50 mL	05030

For a complete list of related products, including specialized cell culture and storage media, matrices, antibodies, cytokines and small molecules, visit www.stemcell.com/hPSCworkflow or contact us at techsupport@stemcell.com.