

# CONSISTENTLY GENERATE hPSC-DERIVED VENTRICULAR CARDIOMYOCYTES

## STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit

Efficiently and reproducibly generate functional, phenotypically pure ventricular cardiomyocytes from human pluripotent stem cells (hPSCs) for use in downstream applications such as disease modeling, drug discovery, and cardiotoxicity screening. The STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit consists of defined, serum-free media optimized for a standardized, 15-day differentiation protocol (Figure 1). Achieve robust differentiation of hPSCs into ventricular cardiomyocytes (Figure 2), which 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 #85850), mTeSR™ Plus (Catalog #100-0276), TeSR™-AOF (Catalog #100-0401), or TeSR™-E8™ (Catalog #05990), and compatible with multiple human embryonic stem (hES) and induced pluripotent stem (hiPS) cell lines.

### Complete Workflow

The STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit 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 differentiation, hPSC-derived ventricular cardiomyocytes can be maintained long-term using STEMdiff™ Cardiomyocyte Maintenance Kit, which enables the standardized harvesting of hPSC-derived ventricular 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 STEMdiff™ Cardiomyocyte Freezing Medium to maintain the viability of cardiomyocytes after thawing.

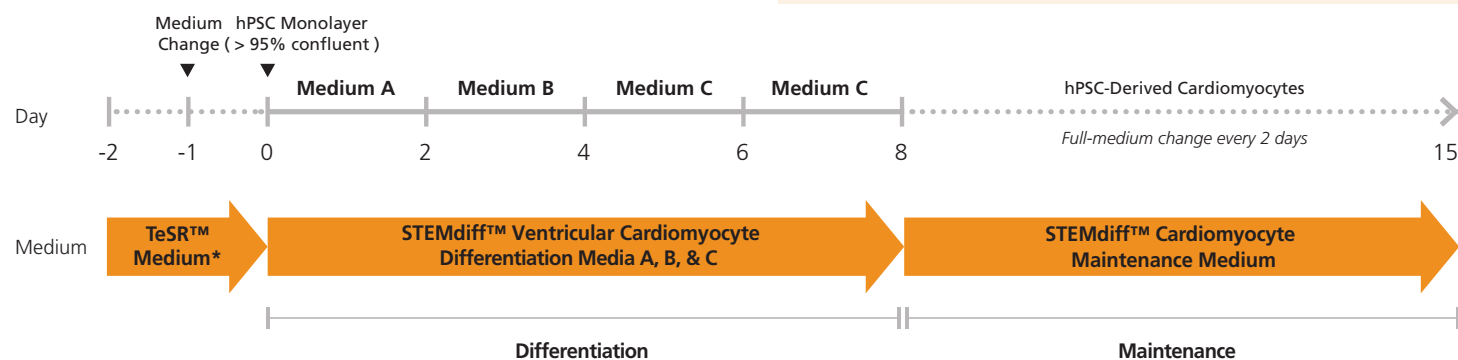
### Why Use STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit?

Support the entire hPSC-derived ventricular cardiomyocyte workflow.

Follow a simple monolayer protocol to produce ventricular cardiomyocytes in 15 days.

Generate over 50 million ventricular cardiomyocytes expressing cTnT.

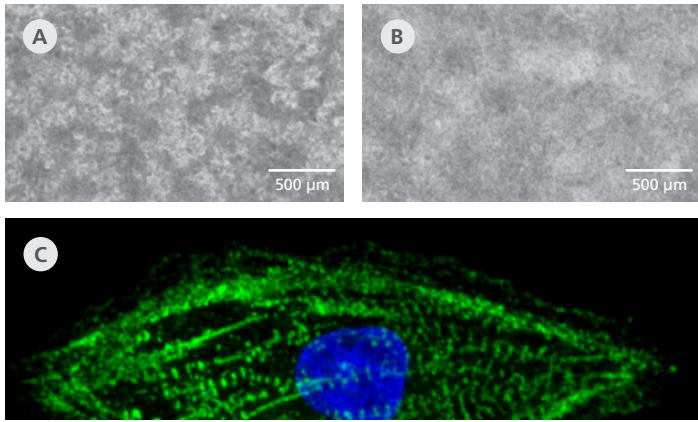
Minimize variability for robust performance across multiple hPSC lines.



\*mTeSR™1, mTeSR™ Plus, or TeSR™-E8™

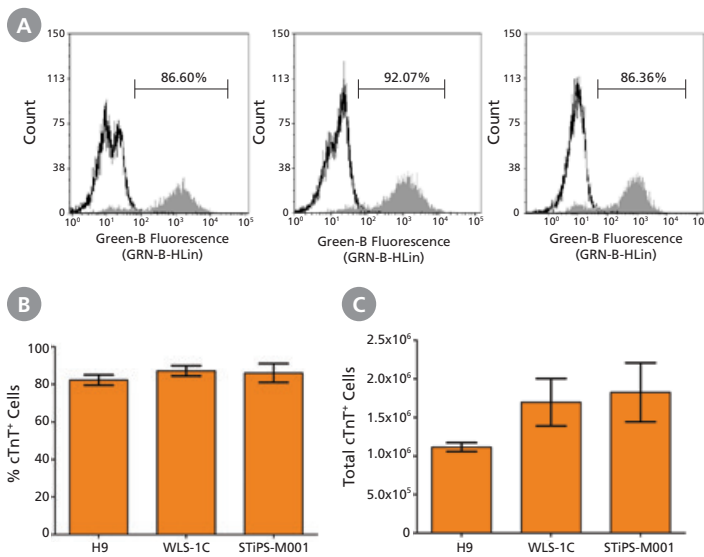
**Figure 1.** Ventricular Cardiomyocyte Differentiation Protocol

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 TeSR™ medium. 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™ Ventricular 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™ Ventricular Cardiomyocyte Differentiation Basal Medium containing Supplement B). On days 4 and 6, full medium changes are performed with fresh Medium C (STEMdiff™ Ventricular 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 monolayer of beating ventricular cardiomyocytes that can be harvested as early as day 15.



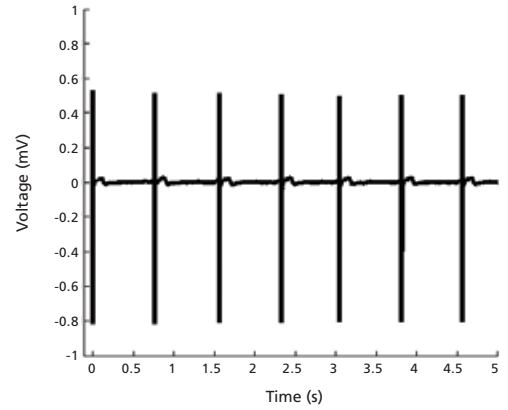
**Figure 2. Morphology of hPSC-Derived Ventricular Cardiomyocytes**

Representative images of (A) hES (H9) cells and (B) hiPS (WLS-1C) cells on day 15 of differentiation to cardiomyocytes using the STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit. Differentiated cells exhibit typical ventricular 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™ Ventricular Cardiomyocyte Differentiation Kit and stained with cTnT (green) and DAPI (blue).



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

hES and hiPS cells were cultured for 15 days in single wells of 12-well plates using the STEMdiff™ Ventricular 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 Ventricular Cardiomyocytes Exhibit a Robust and Stable Electrical Profile**

Microelectrode array (MEA) voltage recordings of ventricular cardiomyocytes (day 27) derived from human pluripotent stem cells generated and maintained with the STEMdiff™ Ventricular Cardiomyocyte Differentiation and Maintenance Kits. The hPSC-derived ventricular 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™ Ventricular Cardiomyocyte Differentiation Kit |  | 1 Kit  | 05010   |
|   | STEMdiff™ Cardiomyocyte Differentiation Basal Medium                 | 380 mL | 05011   |
|   | STEMdiff™ Ventricular Cardiomyocyte Differentiation 10X Supplement A | 10 mL  | 05012   |
|   | STEMdiff™ Ventricular Cardiomyocyte Differentiation 10X Supplement B | 10 mL  | 05013   |
|   | STEMdiff™ Ventricular Cardiomyocyte Differentiation 10X Supplement C | 20 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](http://www.stemcell.com/hPSCworkflow) or contact us at [techsupport@stemcell.com](mailto:techsupport@stemcell.com).

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