

Product No. : 13166-05, 15345-24, 15346-14

AscleStem® Cardiomyocyte Differentiation Medium

Product description

AscleStem® Cardiomyocyte Differentiation Medium is serum free medium for production of differentiated cardiomyocytes from hPSCs. We have differentiated cardiomyocyte from hPSCs cultured on Vitronectin using Essential 8™ Flex and other substrate/medium (Substrate: Matrigel®, Geltrex™, rhLaminin-521, iMatrix-511/ Medium: TeSR E8, StemFit™ AK02N, NutriStem™). Cardiomyocyte differentiation efficiency of hPSCs depends on experimental conditions such as cell lines and culture conditions. We recommend to start by optimization of the protocol, especially concentration of CHIR99021 and density of cells, in small scale for good differentiation efficiency.

Material Required

Material	Product No.	Manufacturer	Product Name
CHIR99021	21068-74	Nacalai Tesque	AscleStem® CHIR99021 Solution(10mM), Animal-Free
IWP-2	21236-64	Nacalai Tesque	AscleStem® IWP-2 Solution(5mM), Animal-Free
SB 431542	21235-74	Nacalai Tesque	AscleStem® SB 431542 Solution(10mM), Animal-Free
Estradiol	21237-25	Nacalai Tesque	AscleStem® Estradiol Solution(1mM), Animal-Free
ROCK Inhibitor	(e.g.) 18190-96	Nacalai Tesque	10mmol/L-Y-27632 Solution
	(e.g.) A2644501	Thermo Fisher Scientific	RevitaCell™

*Require additional reagents depending on protocol

Protocol

[Complete Medium Preparation]

1. Thaw Supplement A for C.D. Medium and Supplement B for C.D. Medium at 4°C.
2. Mix the following components:

AscleStem® Cardiomyocyte Differentiation Medium	500 mL
Supplement A for C.D. Medium	11 mL
Supplement B for C.D. Medium	5 mL

After preparation of Complete Medium, store it at 4°C.

◆ Cardiomyocyte differentiation in monolayer protocol (Sample Procedure)

1. (Day -4).
Instead of 0.5 mM EDTA/PBS, use ACCUMAX™ to generate single cell suspension. Resuspend the cells with iPS Cell culture medium supplemented with ROCK Inhibitor and seed them on Substrate-coated plate at the density of 5,000~10,000 cell/cm² (clone dependent).
2. (Day -3, -2, -1).
Change the medium with iPS Cell Culture medium every day. Use enough volume to supplement the cells (e.g. 750uL for 24-well plate, 1.5 mL for 12-well plate, and 3 mL for 6-well plate).
3. (Day 0).
Change the medium with Complete Medium supplemented with CHIR99021. (We recommend starting with a CHIR99021 concentration of 8 uM) Use enough volume to supplement the cells (e.g. 750 uL for 24-well plate, 1.5 mL for 12-well plate, and 3 mL for 6-well plate).

4. (Day 2).

Change the medium with Complete Medium supplemented with 5 μ M IWP 2, 5 μ M SB 431542, 100 nM Estradiol.
Use enough volume to supplement the cells (e.g. 750 μ L for 24-well plate, 1.5 mL for 12-well plate, and 3 mL for 6-well plate).

5. (Day 4 and every other day thereafter).

Change the medium with Complete Medium supplemented with 100 nM Estradiol. Use enough volume to supplement the cells (e.g. 750 μ L for 24-well plate, 1.5 mL for 12-well plate, and 3 mL for 6-well plate).

Spontaneous beating is observed around day 7.

◆ **Cardiomyocyte differentiation in EB formation protocol** (Sample Procedure)

[EB formation Medium Preparation]

For general applications, we recommend DMEM/F12 (Nacalai Tesque, code:11581-15) supplemented with 15% KnockOut™ Serum Replacement (Gibco™, code:10828028), 1x MEM-Non essential amino acids (Nacalai Tesque, code:06344-14), 0.45 mM Monothioglycerol (Sigma, code:M6145), 5 μ M Y-27632.

Alternatively, you can also use commercially available feeder-free media supplemented with 5 μ M Y-27632. We confirmed successful differentiation using ReproFF, mTeSR™1, Essential 8™ and NutriStem™.

After preparation of EB formation Medium, store it at 4°C.

1. (Day -1).

Generate small clumps of hiPSCs just like you passage your hiPSCs in routine culture. Suspend the small clumps in EB formation medium and start suspension culture using flask/dish/plate with Ultra-Low attachment surface (Corning). We routinely collect the iPSC clumps from one 10 cm dish, suspend with 10 mL EB formation medium and start suspension culture culturing in two 25 cm² flasks, each containing 5 mL of cell suspension.

2. (Day 0).

Change the medium on the next day (approximately 24 hours after starting suspension culture). Collect the small embryoid body (EB) to the centrifuge tube and incubate in the CO₂ incubator for 20 minutes to settle the cell clumps. Aspirate the supernatant containing dead cells and add 5 mL of pre-warmed IMDM. Settle the cell clumps and aspirate the supernatant again. Suspend the cells with 5 mL Complete Medium supplemented with CHIR99021 (We recommend starting CHIR99021 concentration of 4 μ M). Wash the 25 cm² flask once with 2 mL IMDM and return the cell suspension.

3. (Day 2).

Change the medium 48 hours after the first medium change (step 2). Collect the cell clumps and wash the cells twice as described in step 2. Suspend the cells with 5 mL Complete Medium supplemented with 5 μ M IWP2, 5 μ M SB 431542, 100 nM Estradiol. Wash the 25 cm² flask twice with 2 mL IMDM and return the cell suspension.

4. (Day 5 and every 3 days thereafter*).

Change the medium 3 days after step 3. Collect the cell clumps as described in step 2, suspend the cells with 5 mL Complete Medium supplemented with 100 nM Estradiol and return the EB suspension to 25 cm² flask.

Spontaneous beating is observed around day 11.

Storage

Storage temperature is stated on the product label.

Expiration

Expiration date is stated on the product label.

Component

Product No.	Product	Size
13166-05	AscleStem® Cardiomyocyte Differentiation Medium	500 mL
15345-24	Supplement A for C.D. Medium	11 mL
15346-14	Supplement B for C.D. Medium	5 mL

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