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Human Cardiomyocyte Differentiation Medium Kit

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Product Description

The Human Cardiomyocyte Differentiation Medium Kit is a ready-to-use, defined, serum-free medium system designed to efficiently differentiate human embryonic stem cells (hESCs) into cardiomyocyte-like cells in a monolayer culture format.

The kit includes stage-specific media that support cardiac lineage induction and specification, promoting robust and reproducible differentiation. Under recommended culture conditions, spontaneously beating cardiomyocyte networks are typically observed approximately 9 days after differentiation is initiated (Day 0), following the pre-differentiation cell expansion and preparation phase. The formulation minimizes batch-to-batch variability and supports scalable, consistent differentiation workflows.

Key Features

- Stepwise differentiation from mesoderm induction to cardiomyocyte specification and functional maturation
- Spontaneous cardiomyocyte contractions are typically observed around Day 9 of differentiation (Day 0 = initiation of differentiation).
- Supports expression of key cardiac markers, including cTnI and a-actinin

Kit Components

Kit Component	Part No.	Quantity	Storage Conditions
Basal Medium 6	BM006-100	100 ml	2-8°C
HCDMK - Supplement 1	TM213-1	96 µl	-20°C
HCDMK - Supplement 2	TM213-2	90 µl	-20°C
HCDMK - Supplement 3	TM213-3	96 µl	-20°C
B27 Supplement (50X)	SM001-2	2 ml	-20°C
B27 Supplement (50X), without Insulin	SM007-2	2 ml	-20°C
HCDMK - Selection Medium	TM213-4	25 ml	2-8°C

Storage and Stability

- Store each component according to the storage conditions listed above.
- Freshly prepared complete medium is recommended for optimal performance.

Before You Start

Successful cardiomyocyte differentiation depends on starting cell quality, confluency, coating consistency, and timing.

For best results:

- Use healthy hESCs maintained under feeder-free pluripotent stem cell culture conditions.
- Cells should be actively growing and show typical pluripotent stem cell morphology.
- Cells should be free of mycoplasma contamination.
- Culture cells for at least three passages after thawing or recovery before starting differentiation.
- Begin differentiation when cells reach approximately 90-95% confluency with healthy morphology.
- Use evenly coated culture plates and avoid harsh washing or pipetting.
- Follow the medium change schedule carefully, as timing can affect differentiation efficiency.

Differentiation efficiency may vary depending on cell source, passage number, seeding density, and culture history. Minor optimization may be required when using a new cell line.

Additional required materials *(not included in kit)*

- Human embryonic stem cells (hESCs)
- Stem cell growth medium (mTeSR™)
- 1X DPBS, No Ca^{2+}, No Mg^{2+}(CH110)
- 3DCelMatrix™ (TM076)
- DMEM/F-12 (1:1) Medium (TM510)

Stage-Specific Medium Overview

Stage	Medium	Purpose
Stage 1	Medium 1	Mesoderm induction
Stage 2	Medium 2	Cardiac lineage specification
Stage 3	Medium 3	Early cardiomyocyte induction
Stage 4	Medium 4	Early cardiomyocyte maturation
Stage 5	Medium 5	Cardiomyocyte maintenance and continued functional maturation
Stage 6	Medium 6	Cardiomyocyte selection and enrichment

Medium Preparation

Prepare complete media in a biosafety cabinet. Thaw each reagent at room temperature (15–25°C) or overnight at 2–8°C, then mix gently before use.

Important: Freshly prepared complete medium is recommended for optimal performance. Conditioned medium required for Medium 3 preparation should be collected during the Day 3–5 stage as described in the Detailed Protocol.

Medium	Preparation
Medium 1	11.66 ml Basal Medium 6 + 240 µl B27 without insulin + 96 µl Supplement 1
Medium 2	11.76 ml Basal Medium 6 + 240 µl B27 without insulin
Medium 3	5.34 ml conditioned medium + 6.45 ml fresh Basal Medium 6 + 120 µl B27 without insulin + 90 µl Supplement 2
Medium 4	11.76 ml Basal Medium 6 + 240 µl B27 without insulin
Medium 5	11.76 ml Basal Medium 6 + 240 µl B27
Medium 6	23.42 ml Selection Medium + 480 µl B27 without insulin + 96 µl Supplement 3 Prepare according to the validated selection medium formulation before use

Note: Addition of supplements may make the complete medium appear slightly opaque. This is expected. Antibiotics may be added if required by the user's culture workflow.

Simplified Differentiation Workflow

Timeline	Medium	Step
Day -4 to Day 0	Stem cell growth medium	Prepare and expand healthy hESCs until cells reach approximately 90-95% confluency.
Day 0-1	Medium 1	Start mesoderm induction.
Day 1-3	Medium 2	Continue early cardiac lineage specification.
Day 3-5	Medium 3	Support cardiac specification and early cardiomyocyte induction.
Day 5-9	Medium 4	Support early cardiomyocyte maturation.
Day 9-15	Medium 5	Maintain developing cardiomyocytes and support functional maturation. Spontaneous contractions are typically observed during this stage.
Day 15-19	Medium 6	Perform cardiomyocyte selection and enrichment.
Day 19+	Medium 5	Maintain enriched cardiomyocyte cultures.

Detailed Protocol

The following protocol is optimized for generating human cardiomyocytes in a 6-well plate format.

Unless otherwise specified, all cultures were maintained at 37°C with 5% CO_2.

Day -4 to Day 0: Recovery and Preparation of hESCs for Cardiomyocyte Differentiation

Before initiating differentiation, passage and culture the stem cells for at least three passages to ensure the cells are fully recovered, healthy, free of mycoplasma contamination, and exhibit stable growth and doubling rates.

Initiation of human embryonic stem cell (hESC) culture

1. Prepare a 3DCelMatrix™ coated 6-well plate.
    a. Coat culture vessels with 1% 3DCelMatrix™ using cold DMEM/F-12 Medium.
    b. Incubate coated vessels at 37°C for 2 hours prior to use.
2. Add 2.0 ml of appropriate stem cell growth medium (mTeSR™).
3. Seed hESCs onto the coated 6-well plate at approximately 10-20% confluency.
4. Replace medium daily and maintain cells until they reach approximately 90–95% confluence with healthy morphology. Once the cells meet these criteria, initiate mesoderm induction (Day 0).
   Note: Day 0 is defined as the initiation of mesoderm induction. The duration of the cell recovery and expansion phase may vary depending on cell growth characteristics and culture conditions.

Day 0–1: Mesoderm Induction

Prepare Medium 1 according to the Medium Preparation table prior to use.

1. Aspirate culture medium and gently wash cells once with 1X DPBS, No Ca^{2+}, No Mg^{2+} to remove residual factors that may interfere with differentiation.
2. Add 2.0 ml of **Medium 1** per well.
3. Incubate cells in **Medium 1** for approximately 24 hours.

Expected observations

- A transient reduction in cell density may be observed during early lineage commitment.
- Some cell death may be observed and is expected during early differentiation.
- Cells may exhibit changes in morphology consistent with mesoderm induction.

Note: Differentiation timing and morphology may vary depending on cell source, passage number, starting confluency, and culture conditions.

Day 2–3: Early Lineage Stabilization

Prepare Medium 2 according to the Medium Preparation table prior to use.

1. Aspirate **Medium 1** and gently wash once with 1X DPBS, No Ca^{2+}, No Mg^{2+}.
2. Add 2.0 ml of **Medium 2** per well.
3. Maintain cells in **Medium 2** until Day 3.

Expected observations

- Cells may begin forming a more uniform monolayer with reduced pluripotency characteristics.

Day 3–5: Cardiac Specification

Prepare Medium 3 according to the Medium Preparation table prior to use.

1. Collect 5.34 ml of conditioned medium and set aside under sterile conditions for **Medium 3** preparation.
2. Aspirate **Medium 2** and gently wash once with 1X DPBS, No Ca^{2+}, No Mg^{2+}.
3. Add 2.0 ml of **Medium 3** per well.
4. Maintain cells in **Medium 3** until Day 5, with medium changes every 48 hours.

Expected observations

- Changes consistent with early cardiac lineage commitment may be observed during this phase.
- By the end of Day 5, cells may exhibit increased density and a more compact epithelial-like morphology.

Day 5–9: Early Cardiomyocyte Emergence

Prepare Medium 4 according to the Medium Preparation table prior to use.

1. Aspirate **Medium 3** and gently wash once with 1X DPBS, No Ca^{2+}, No Mg^{2+}.
2. Add 2.0 ml of **Medium 4** per well.
3. Maintain cells in **Medium 4** until Day 9, with medium changes every 48 hours.

Expected observations

- Early contractile activity may begin toward the end of this stage in some wells.

Day 9–15: Cardiomyocyte Maturation Onset

Prepare Medium 5 according to the Medium Preparation table prior to use.

1. Continue culture in **Medium 5**.
2. Replace **Medium 5** every 48 hours.

Expected observations

- Spontaneous cardiomyocyte contractions are typically observed beginning around Day 9 of differentiation.
- Contractile activity may become more pronounced over time, with beating clusters progressively increasing in size and synchronization.

Day 15-19: Selection

Prepare Medium 6 according to the Medium Preparation table prior to use.

1. Aspirate **Medium 5** and gently wash once with 1X DPBS, No Ca^{2+}, No Mg^{2+}.
2. Add 2.0 ml of **Medium 6** per well.
3. Replace medium every 48 hours

Day 19+

Prepare Medium 5 according to the Medium Preparation table prior to use

1. Aspirate **Medium 6** and gently wash once with 1X DPBS, No Ca^{2+}, No Mg^{2+}.
2. Add 2.0 ml of **Medium 5** per well.
3. Replace medium every 48 hours.

Expected observations

- Robust, synchronous beating networks may be established.
- Cells may exhibit organized sarcomeric structures and functional cardiac properties suitable for downstream assays (drug testing, calcium flux, electrophysiology).

Troubleshooting Guide

Observation	Possible Cause	Recommended Action
Low cell attachment after seeding	Uneven coating, stressed cells, or harsh dissociation	Confirm even plate coating. Use healthy cultures and avoid harsh dissociation. Allow cells to attach undisturbed after seeding.
Uneven monolayer before Day 0	Uneven seeding or inconsistent coating	Distribute cells evenly after seeding and avoid excessive plate movement during attachment.
Cells are under-confluent on Day 0	Seeding density was too low or cells grew slowly	Do not start differentiation too early. Continue culture until cells reach approximately 90-95% confluency.
Cells are over-confluent on Day 0	Seeding density was too high or cells were cultured too long	Passage and reseed cells for a new differentiation run. Reduce seeding density in future runs.
Excessive cell death after Day 0	Poor starting cell quality, harsh handling, or incorrect medium preparation	Use healthy cultures, confirm medium preparation, wash gently, and avoid allowing cells to dry.
Delayed or no beating	Suboptimal cell quality, confluency, timing, or medium preparation	Confirm cell health, mycoplasma status, medium preparation, and timing. Optimize seeding density if needed.
Incomplete selection	Selection started too early or Medium 6 preparation was incorrect	Confirm Medium 6 preparation and begin selection only after cardiomyocyte-like morphology and beating are observed.
Cells detach during medium changes	Pipetting or wash steps were too forceful	Add and remove media slowly along the side wall of the well. Avoid direct pipetting onto the cell layer.

If poor differentiation is observed repeatedly, confirm cell line health, passage number, mycoplasma status, coating quality, medium preparation, and incubator conditions before repeating the protocol.