Data Sheet

HL-1 Cardiac Muscle Cell Line

SCC065

Pack Size: ≥ 1x10^6 viable cells/vial

Store in liquid nitrogen.

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures. Not for human or animal consumption.

Background

The HL-1 Cardiac Muscle Cell Line is an immortalized mouse cardiomyocyte cell line able to continuously divide and spontaneously contract while maintaining a differentiated cardiac phenotype. HL-1 can be serially passaged without losing its differentiated cardiac myocyte phenotype, including morphological, biochemical, and electrophysiological properties. HL-1 is used in a variety of model systems to address questions of cardiac biology at the cellular and molecular levels.

Spontaneous contraction (for example, beating) of HL-1 may not be observable by bright-field microscopic inspection as the beating is extremely subtle. The beating phenotype may be observed by using a fluorescent calcium flux dye, Fluo-8®. Please refer to the protocol for the Fluo-8® functional assay.

Source

This product contains genetically modified organisms. The HL-1 line was derived from AT-1 subcutaneous tumor excised from an adult female C57BL/6J mouse. The parental AT-1 line was originally derived from an atrial tumor growing in a transgenic mouse in which expression of the SV40 large T-antigen was targeted to atrial cardiomyocytes via the atrial natriuretic factor (ANF) promoter.

Quality Control Testing

- Each vial contains ≥ 1x10^6 viable cells.
- Cells tested negative for infectious diseases by the Mouse Essential CLEAR panel PCR panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.
- Functional Assay: Fluo-8® calcium dye fluorescent based assay for detecting changes in intracellular calcium.

Storage and Handling

The HL-1 Cardiac Muscle Cell Line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Representative Data

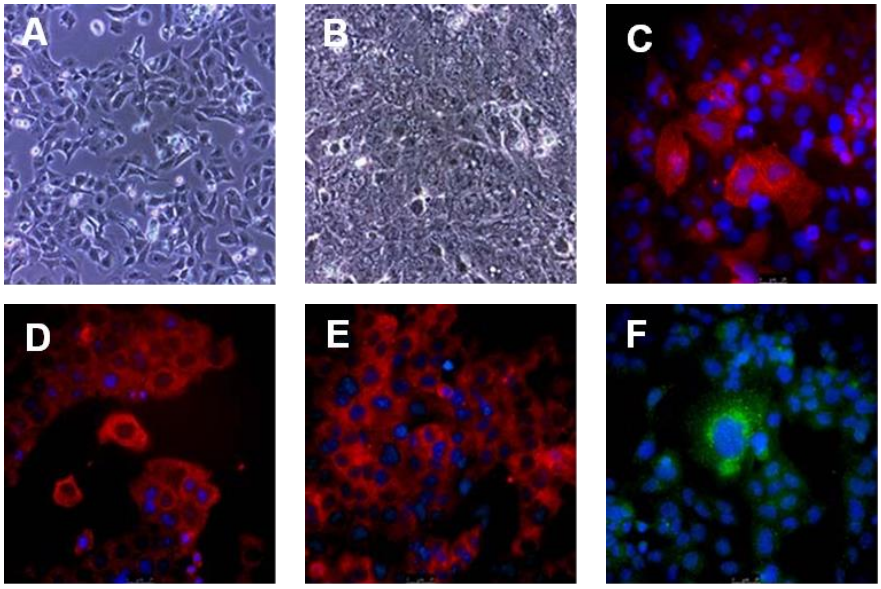

Figure 1. Cell morphology and immunocytochemistry staining of HL-1 cardiac muscle cell line. Bright-field images of HL-1 cells (**A**) one and (**B**) three days after thawing in a T25 flask. (**C**) HL- 1 cells express α-actinin, (**D**) Titin, (**E**) Myosin (MF-20), and (**F**) ANF.

Protocols

Gelatin/Fibronectin ECM Coating of Flasks

1. Add 10 mL of 0.1% Gelatin Solution (ES-006) to 40 mL of Ultrapure Water in a sterile 50 mL conical tube. Final concentration = 0.02% Gelatin Solution
2. Add 250 µL Fibronectin (1 mg/mL, F-1141) to the 50 mL 0.02% Gelatin Solution. Final concentration = 5 µg/mL Fibronectin
3. Sterilize filter using 0.2 µm filter (SE1M179M6 or SCGP00525).
4. Aliquot 6 mL of Gelatin/Fibronectin ECM Solution into sterile 15 mL centrifuge tubes. Label and store at –20 °C.
5. Coat flasks with the Gelatin/Fibronectin ECM mixture (3 mL for T25, 6 mL for T75 or 15 mL for T225 flasks) at 2-8 °C overnight or at 37 °C for a minimum of 1 hour. Flasks may be coated 5-6 days in advance and stored at 2-8 °C.

Preparation of Medium

Preparation of 100X (10 mM) Stock Solution of (+)- Norepinephrine [(+)-arterenol] (A0937)

1. Prepare 50 mL of 30 mM L-Ascorbic Acid (A7506) by dissolving 0.264 g L-Ascorbic acid in 50 mL of distilled water.
2. Add 160 mg Norepinephrine to the 50 mL of 30 mM L-Ascorbic acid solution. Sterile filter using 0.2 µm filter.
3. Aliquot the 100X Norepinephrine stock solution to sterile microtubes and store at –20 °C for later use.
4. Wrap 100X Norepinephrine stock solution aliquots in aluminum foil to protect them from light. Minimize freeze-thaw cycles. Aliquots stored at –20 °C may be used for up to 1 month.

Preparation of HL-1 Expansion Medium (10% FBS; 500 mL). Scale according to the volumes required.

Component	Quantity	Final Concentration	Cat. No.
Claycomb Basal Medium	435 mL		51800C
HL-1 Qualified FBS	50 mL	10%	TMS-016-B
Norepinephrine (10 mM, 100X)	5 mL	0.1 mM	A0937
L-Glutamine, 200 mM	5 mL	2 mM	TMS-002-C
Penicillin/Streptomycin, 100X	5 mL	1X	TMS-AB2-C

Wrap HL-1 Expansion Medium in aluminum foil as the supplemented medium is extremely light sensitive. Store at 4 °C. HL-1 Expansion Medium should be used within two weeks after preparation.

Thawing the Cells

The HL-1 cells should always be maintained at high cell density. Frozen vials should be thawed into a T25 flask. Culture medium should be exchanged daily. Cells should be passaged at 1:3 split ratio and only when they are 100% confluent. Do not split cells beyond 1:3 as this may cause cells to dedifferentiate.

1. Do not thaw the cells until the Gelatin/Fibronectin coated tissue culture flasks and recommended expansion medium are on hand.
2. Remove the vial of frozen HL-1 cells from liquid nitrogen and thaw in a 37 °C water bath. Closely monitor the cells to ensure complete, but not overthawing. Maximum cell viability depends on the rapid and complete thawing of frozen cells.

   Important: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol.
4. In a laminar flow hood, use a 1- or 2-mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of HL-1 Cell Expansion Medium to the 15 mL conical tube.

   Important: Do not add the entire volume of medium all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   Important: Do not vortex the cells.
7. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible.
9. Resuspend the pelleted cells into 10-15 mL of HL-1 Expansion Medium.
10. Transfer the cell suspension to a Gelatin/Fibronectin coated T25 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.
12. The next day, exchange the medium with 10-15 mL of fresh HL-1 Cell Expansion Medium. Exchange with fresh medium every day.
13. When the cells are 100% confluent, they can be dissociated with 0.05% Trypsin in 0.02% EDTA (T3924) and further passaged or, alternatively, frozen for later use.

    Note: It is absolutely critical to use Trypsin-EDTA solution.
14. Cells should be passaged at 1:3 ratio into the appropriate Gelatin/Fibronectin ECM coated flasks. Do not passage cells beyond 1:3 split ratio as this may cause cells to dedifferentiate.

Cryopreservation of Cells

The HL-1 Cardiac Cell Line may be frozen in 95% HL-1 Qualified FBS (TMS-016-B) containing 5% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

Protocol for Detection of Spontaneous Calcium Fluctuations in Confluent HL-1 Cells

Please have on hand a 100% confluent T25 flask of HL-1 cells. Please refer to protocol above on culture of HL-1 cells.

1. Prepare a Gelatin/Fibronectin coated 24-well tissue culture plate with 0.5 mL per well of ECM. Please refer to Gelatin/Fibronectin ECM coating of flasks in the protocol section above.
2. Prepare fresh HL-1 Expansion Medium. Please refer to protocol above for the formulation.
3. Dissociate the confluent layer of HL-1 cells with 0.5% Trypsin in 0.02% EDTA.

   Note: It is absolutely critical to use Trypsin EDTA (T3924).
4. Count and dilute 3 million cells into 24 mL of fresh HL-1 Expansion Medium. Dispense 1 mL per well of the cell suspension into each well of the coated 24-well plate. This corresponds to approximately 125,000 cells per well. Swirl the plate to ensure even plating of the cells.
5. Allow HL-1 cells to grow to 100% confluency with media changes every day. It will typically take 3-4 days for the cells to be 100% confluent. Alternatively, the cells can be plated at a higher cell density but must be monitored to ensure that cells reach confluency but are not overgrown. It is important that fresh media is exchanged daily.

On the day of the assay (for example, when cells reach 100% confluency):

6. Prepare a 100X Fluo-8® AM stock solution (500 µM): Add 100 µL of DMSO to 1 vial (50 µg) of Fluo-8® AM (AAT Bioquest, 21081). Final concentration = 500 µM. Store at –20 °C in a tinfoil wrapped vial until ready to use.
7. Aspirate the growth medium from the 24-well plate containing confluent HL-1 cells. Rinse each well to be stained with 1 mL of Hepes Buffered Tyrode's Solution (ThermoFisher, 50151910) OR Hanks' Buffer with 20 mM Hepes (HHBS) (AAT Bioquest, 20011).
8. Prepare a 5 µM Fluo-8® working solution by diluting 15 µL of the 500 µM Fluo-8® AM stock solution into 1.5 mL of Tyrode's Solution.
9. Aspirate the wells to be stained and replace with 0.5 mL per well of 5 µM Fluo-8® solution.
10. Incubate at 37 °C for 1 hour.
11. After 1 hour, aspirate the staining solution. Rinse each well with 1 mL per well of Tyrode's Solution.
12. Aspirate and add 1 mL per well Tyrode's Solution to each well.
13. Immediately view the cells using a fluorescent microscope equipped with a 20X objective and a GFP or FITC filter set. With the fluorescent microscope, spontaneous contraction of HL-1 cells can be readily observed and videotaped.

References

1. Proc. Natl. Acad. Sci. USA. 95(6): 2979-2984.
2. Am. J. Physio. Heart Circ. Physiol 286(3): H823-9.

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