Data Sheet

Assay Ready HL-1 Mouse Cardiac Muscle Cell Line

SCCAR10065-1VL

Pack size: 1 vial

Store in Liquid Nitrogen

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Assay ready cells cannot be expanded or passaged. Please see terms of use agreement below.

Background

Assay ready HL-1 cells are presented in a format that enables them to be thawed and grown directly in a 96-well plate for cell viability or other assays without the requirement of prior expansion in culture flasks or dishes.

The HL-1 mouse cardiac muscle cell line is an immortalized cardiomyocyte line able to continuously divide and spontaneously contract while maintaining a differentiated cardiac phenotype. HL-1 cells retain morphological, biochemical, and electrophysiological properties of adult cardiomyocytes. HL-1 cells are widely used to address questions of cardiac biology at the cellular and molecular levels.

Spontaneous contraction (for example, beating) of HL-1 cells may not be observable by brightfield microscopy 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.

Assay ready HL-1 cells are suitable for multi-well assays. One vial of assay-ready HL-1 cells yield confluent monolayers in a 96-well plate by day three after thaw.

Source

HL-1 cardiomyocyte cells were isolated from AT-1 subcutaneous tumor excised from an adult female C56BL/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.

Short Tandem Repeat

M1-1: 17	M1-2: 19	M2-1: 16	M3-2: 14
M4-2: 20.3	M5-5: 17,18	M6-4: 18	M6-7: 12,17
M7-1: 26.2	M8-1: 16	M11-2:16	M12-1: 16,17
M13-1:17	M15-3: 21.3	M17-2:15	M18-3: 16
M19-2:13	MX-1: 28		

Quality Control Testing

- Each vial contains ≥ 2.8 x 10^6 viable cells at thaw.
- Assay Ready HL-1 cells are verified to be of mouse origin and negative for human, rat, Chinese hamster, Golden Syrian hamster, and non-human primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells tested negative for infectious disease against a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

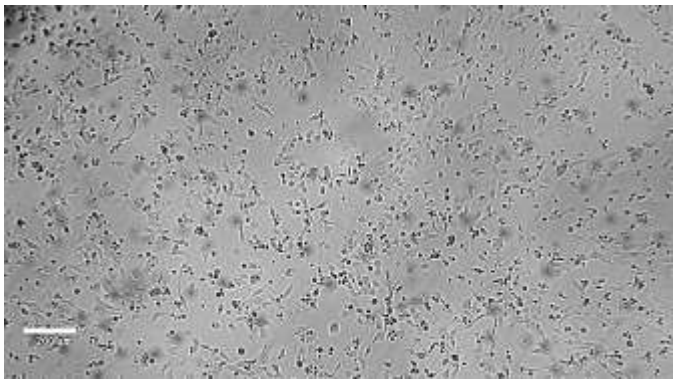
Storage and Handling

HL-1 cells should be stored in liquid nitrogen. Upon recovery, the cells can be plated directly into one 96-well at a density of 30,000 live cells/well in their expansion medium. Treatments and viability assays can be performed 48-hour post plating the cells. **Do not expand, passage, or cryopreserve these cells after recovery.**

Representative Data

Figure 1: Approximately timeline for performing cell assays.

A



B

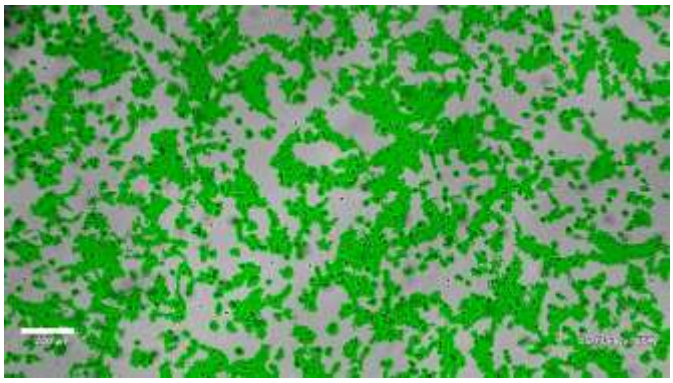


Figure 2. (**A, B**) After thaw, HL-1 assay ready cells are plated at a density of 30,000 viable cells/well in a 96-well plate. 24 hours post plating, cells are ~50% confluent as assessed by the Millicell® Digital Cell Imager (MDCI10000, 10X magnification). Cells are ~100% confluent by Day 3 post plating and are amenable to physiological assays (image not shown).

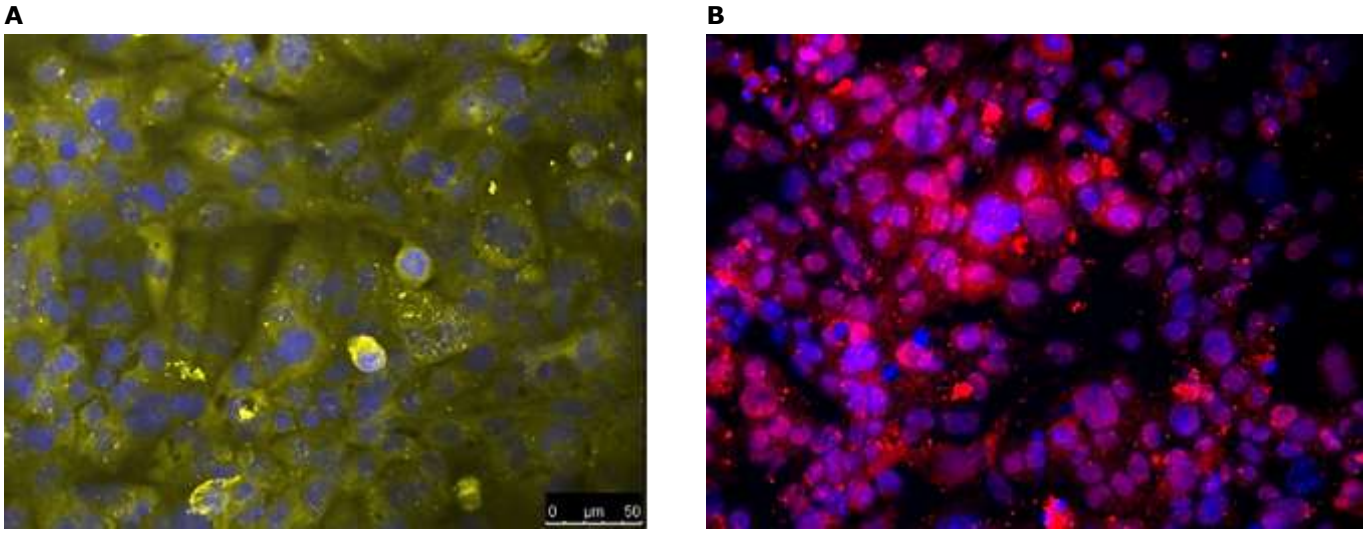


Figure 3. (**A**). Assay Ready HL-1 cells express myosin heavy chain 4 Factor (MYH4, MABT847). (**B**) Assay Ready HL-1 cells express Atrial Natriuretic Factor (ANF, AB5940).

Protocols

Gelatin/fibronectin ECM Coating of Plates

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. Sterile filter using 0.2 µm filter (SE1M179M6 or SCGP00525).
4. Aliquot 10 mL of Gelatin/fibronectin ECM Solution into sterile 15 mL centrifuge tubes. Label and store at −20 °C.
5. Coat plate with the Gelatin/fibronectin ECM mixture (10 mL for 96-well plate, 100 µL/well) at 2-8 °C overnight or at 37 °C for a minimum of 1 hour. Plates may be coated 5-6 days in advance and stored at 2-8 °C.

Preparation of Medium

6. Preparation of 100X (10 mM) Stock Solution of (+)− Norepinephrine (+)−bitartrate salt (A0937)
    - Prepare 50 mL of 30 mM L-Ascorbic acid (A7506) by dissolving 0.264 g L-Ascorbic acid in 50 mL of distilled water.
    - Add 160 mg Norepinephrine to the 50 mL of 30 mM L-Ascorbic acid solution. Sterile filter using 0.2 µm filter (SE1M179M6 or SCGP00525).
    - Aliquot the 100X Norepinephrine stock solution to sterile microtubes and store at −20 °C for later use.
    - Wrap 100X Norepinephrine stock solution aliquots in aluminum foil to protect them from light. Minimize freeze-thaws. Aliquots stored at −20 °C may be used for up to 1 month.

7. Preparation of HL-1 Culture Medium (10% FBS, 500 mL). Scale according to the volumes required. Catalog numbers can be purchased at SigmaAldrich.com unless otherwise stated.

Component	Quantity	Final Concentration	Catalogue No.
Claycomb Basal Medium	435 mL	-	51800C
HL-1 Qualified FBS	0 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

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

Thawing the Cells

Physiological assays of HL-1 cells should be done at high cell density. Culture medium should be exchanged daily.

9. Do not thaw the cells until the Gelatin/fibronectin coasted plates and recommended culture medium are on hand.
10. Remove the vial of assay-ready HL-1 cells from liquid nitrogen and incubate in a 37 °C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

    IMPORTANT: Do not vortex the cells.
11. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
12. In a laminar flow hood, use a 1-2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
13. Using a 10 mL pipette, slowly add dropwise 9 mL of HL-1 Culture Medium (Step 7 above, pre-warmed to 37 °C) to the 15 mL conical tube.

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

    IMPORTANT: Do not vortex the cells.
15. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.
16. Decant as much of the supernatant as possible. Steps 13-16 are necessary to remove residual cryopreservative (DMSO).
17. Resuspend the cells in a small volume of HL-1 culture medium (Step 7 above, pre-warmed to 37 °C).
18. Count cells using hemacytometer.
19. Plate onto a 96-well plate at a density of 30,000 viable cells per well in 100 µL volume of HL-1 culture medium.
20. Incubate the cells at 37 °C in a 5% CO_2 humidified incubator.
21. Monitor cell confluency using the Millicell® Digital Cell Imager (MDCI10000). One day after plating, cells should be ~50% confluent.
22. On day 1 (24-hour post plating), cells can be treated with compounds of interest. Physiological assays can be done at confluency (typically 72-hour post-plating).

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

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

1. 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.
2. Aspirate the growth medium from the 96-well plate containing confluent HL-1 cells. Rinse each well to be stained with 50 µL of HEPES-buffered Tyrode's Solution (ThermoFisher, 50-151-910) OR Hanks' Buffer with 20 mM Hepes (HHBS) (AAT Bioquest, 20011).
3. 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.
4. Aspirate the wells to be stained and replace with 0.5 mL per well of 5 µM Fluo-8 solution.
5. Incubate at 37 °C for 1 hour.
6. After 1 hour, aspirate the staining solution. Rinse each well with 50 µL per well of Tyrode's Solution.
7. Aspirate and add 50 µL Tyrode's Solution to each well.
8. 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. Claycomb WC, Lanson NA Jr, Stallworth BS, Egeland DB, Delcarpio JB, Bahinski A, Izzo NJ Jr. 1998. HL-1 cells: a cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte. *Proc Natl Acad Sci USA* 95(6):2979-2984.
2. White SM, Constantin PE, Claycomb WC. 2004. Cardiac physiology at the cellular level: use of cultured HL-1 cardiomyocytes for studies of cardiac muscle cell structure and function. *Am J Physiol Heart Circ Physiol* 286(3):H823-829.

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Document Template 20306518 Ver 6.0

23078730 Ver 1.0, Rev 21JUN2024, AV