SAFC
3050 Spruce Street
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Product Information

Claycomb Medium
without L-glutamine

Catalog No. 51800C

Description
Claycomb Medium, named after Dr. William Claycomb who established the HL-1 cell line, is specifically designed for the growth of murine cardiomyocytes. HL-1 cells are the first cell line established that can maintain the differentiated cardiomyocyte-phenotype and contractile activity *in vitro*. The HL-1 cell line can be used for the study of cardiac cell hypertrophy that follows myocardial infarction, the testing of novel cardiotherapeutic drugs and treatments, the production of high levels of cardiac proteins and the study of mature cardiomyocyte specific genes.

Claycomb Medium, when supplemented with 100 µM norepinephrine, 10% Fetal Bovine Serum (FBS) and 2 mM L-glutamine, will maintain the HL-1 cell line and the mature cardiomyocyte phenotype. Under a light microscope, individual and groups of HL-1 cells can be observed contracting, with the frequency of contractions increasing as the cardiomyocytes reach confluency.

Formulation
The formula for Claycomb Medium is proprietary to SAFC. For additional information please call our Technical Services department.

Precautions
Use sterile technique when handling or supplementing this medium. This product is for research or for further manufacturing use. THIS PRODUCT IS NOT INTENDED FOR HUMAN OR THERAPEUTIC USE.

Storage
Store medium protected from light at 2 to 8°C.

Indications of Deterioration
Medium should be clear and free of particulates and flocculent material. Do not use if medium is cloudy or contains precipitate. Other evidence of deterioration may include color change, pH shift or degradation of physical or performance characteristics.

Supplemented Claycomb Medium

Product Name	ml	Final Concentration
Claycomb Medium	87	
Fetal Bovine Serum	10	10%
Penicillin/Streptomycin	1	100 U/ml:100 µg/ml
Norepinephrine (10 mM stock)	1	0.1 mM
L-Glutamine (200 mM stock)	1	2 mM

- Wrap the Claycomb Medium bottle in aluminum foil, since the medium is extremely light sensitive.
- Supplemented Claycomb Medium is good for two weeks, at which time L-glutamine is replenished.

Norepinephrine [(±)-arterenol], mw 319.3
- Norepinephrine is made up in 30 mM ascorbic acid.
- Make up 100 ml of 30 mM ascorbic acid by adding 0.59 g ascorbic acid to 100 ml of cell culture grade distilled water.
- Add 80 mg norepinephrine to 25 ml of the 30 mM ascorbic acid.
- Filter-sterilize using a 0.2 µm Acrodisc syringe filter.
- Aliquot in 1 ml volumes into sterile microtubes with screw caps, and store at -20°C. This is 10 mM (stock) norepinephrine. Use 1 ml of stock per 100 ml medium for a 0.1 mM final concentration.
- Norepinephrine needs to be made up fresh monthly.

L-Glutamine
- L-Glutamine comes as a 100x solution, and is aliquoted into working volumes and frozen.

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Freezing Medium

- Freezing medium is made up of 95% FBS/5% DMSO.
- This can be stored up to a week at 4°C.

Soybean Trypsin Inhibitor

- Weigh out 25 mg of soybean trypsin inhibitor, and place into a beaker containing 100 ml of Dulbecco's phosphate buffered saline (PBS; Ca^{2+}-free and Mg^{2+}-free) until dissolved.
- Filter-sterilize, using a 0.2 μm syringe filter, into a 100 ml bottle.
- This is good for a month at 4°C.

Pre-coating Flasks

Gelatin/Fibronectin

- Weigh out 0.1 g gelatin and place into a 500 ml glass bottle.
- Add distilled water to the 500 ml mark, and autoclave. This gelatin will go into solution while being autoclaved. The concentration of the gelatin is 0.02%.
- Fibronectin (1 mg/ml) is received in a tube as a liquid. Dilute 1 ml fibronectin in 199 ml of 0.02% gelatin. Mix gently, and immediately aliquot 6 ml into each labeled 15 ml centrifuge tube. Freeze aliquots at -20°C.
- Before culturing cells, coat tissue culture flasks with gelatin/fibronectin (1 ml/T25 or 3 ml/T75 flask). Cap the flasks, and incubate at 37°C for at least an hour.
- Remove the gelatin/fibronectin by aspiration just before adding cells to the flasks.

Culturing Cells

- Cultures are fed (5 ml/T25 flask) with supplemented Claycomb Medium every weekday.
- To avoid feeding the cells on weekends, 10 ml of supplemented Claycomb Medium is added to each T25 flask on Friday afternoons; this medium is not changed until the following Monday morning.

Passaging – Procedure for a 1:2 Split

After the cells first arrive, it is recommended that they be split when they reach confluency.

- Split each of the T25 flasks 1:2, resulting in four T25 flasks. One of these four T25 flasks will be your "working" set of cells.
- When the cells in the remaining three T25 flasks reach confluency three days later, they will be split 1:3, resulting in 3 T75 flasks to be frozen. We recommend freezing away at least 3 T75 flasks.
- Thus, within a week of receiving the HL-1 cells, you should have three cryovials of these cells frozen away for future use.
- It is recommended that cultures be split only after reaching full confluency.
- Rinse each T25 flask briefly with 3 ml of phosphate buffered saline (PBS) warmed to 37°C (use 6 ml for T75) by pipetting the PBS onto the base of the flask (side opposite the cap), trying not to hit cells directly. Rinse gently and remove by aspiration.
- Add 1 ml of pre-warmed 0.05% trypsin/EDTA per T25 flask (3 ml per T75). Incubate at room temperature for 1 minute.
- Remove and add fresh 0.05% trypsin/EDTA. Incubate at room temperature for an additional 2 minutes.
- Examine microscopically and, if cells are still adhered, rap the flask on the benchtop to dislodge remaining cells.
- To inactivate the enzyme, add an equal amount (1 ml per T25) of soybean trypsin inhibitor.
- Transfer cells from the flask into a 15 ml centrifuge tube.
- Rinse the empty flask with 5 ml wash medium (Claycomb Medium containing only 5% FBS and penicillin/streptomycin), and add to the cells already in the 15 ml centrifuge tube.
- Centrifuge at 500×g for 5 minutes.
- Meanwhile, remove the gelatin/fibronectin solution from each T25 flask, and add 4 ml supplemented Claycomb Medium/flask. Set aside.
- Remove the tube containing the HL-cardiomyocytes from the centrifuge. Remove the supernatant by aspiration, and gently resuspend the pellet in 2 ml of supplemented Claycomb Medium.
- Transfer 1 ml of the cell suspension into each of two labeled, gelatin/fibronectin-coated T25 flask. Each flask now contains 5 ml.
- If the cells are passaged on a Friday, use 2x the volume of supplemented Claycomb Medium per flask.

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Freezing

IT IS RECOMMENDED THAT YOU FREEZE 3 OR MORE VIALS AS SOON AS POSSIBLE AFTER RECEIPT OF THE CELLS (please see note under "Passaging"). This allows you to return to this passage, and also protects you in case of contamination.

- We generally freeze the contents of one confluent T75 flask into one cryovial. (When cells are needed, this cryovial is thawed into one T75 flask.)
- Briefly rinse the T75 flask containing the HL-1 culture with 5 ml of PBS warmed to 37°C. Remove by aspiration.
- Transfer 3 ml of 0.05% trypsin/EDTA into the flask.
- Incubate the flask at 37°C for 1 minute.
- Remove the trypsin/EDTA from the flask, and replace with 3 ml of fresh 0.05% trypsin/EDTA.
- Incubate at 37°C for 2 minutes.
- Check under a microscope that cells are dislodged. If not, rap the flask on the benchtop to dislodge any adherent cells.
- Add 3 ml of soybean trypsin inhibitor to the flask, and transfer the 6 ml into a 15 ml centrifuge tube.
- Rinse each empty flask with 8 ml wash medium, and add to the cells already in the 15 ml centrifuge tube. Total volume is now 14 ml.
- Centrifuge tube for 5 minutes at 500×g.
- Remove wash medium by aspiration.
- Gently resuspend each pellet in 1.5 ml of freezing medium (95% FBS/5% DMSO).
- Pipette resuspended cells into a cryovial. Place the cryovial containing the cells into a Nalgene freezing jar containing room temperature isopropanol.
- Immediately place the freezing jar into a –80°C freezer, which allows the cells to freeze at a rate of -1°C/minute.
- Six to twelve hours later, transfer the vial to a liquid nitrogen dewar.

Thawing

- Gelatin/fibronectin-coat a T75 tissue culture flask for at least an hour in a 37°C incubator.
- Remove the gelatin/fibronectin from the culture flask, and replace with 10 ml of supplemented Claycomb Medium. Place this flask back into incubator.
- Transfer 10 ml wash medium into an empty 15 ml centrifuge tube. Incubate tube in a 37°C water bath.
- Quickly thaw the cells in a 37°C water bath (about 2 min), and transfer into the 15 ml centrifuge tube containing the wash medium.
- Centrifuge for 5 minutes at 500×g.
- Remove the tube from the centrifuge and remove the wash medium by aspiration.
- Gently resuspend the pellet in 5 ml supplemented Claycomb Medium, and add to the 10 ml of medium already in the T75 flask.
- Replace the medium with 15 ml of fresh supplemented Claycomb Medium 4 hours later (after cells have attached).

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Ordering Information

Product Name	Vendor	Catalog No.
Claycomb Medium	Sigma-Aldrich	51800C
Fetal Bovine Serum	Sigma-Aldrich	F2442* (Batch 15K284; US origin)
Penicillin-Streptomycin (10^4 U/ml P and 10^4 µg/ml S)	Sigma-Aldrich	P4333
Norepinephrine [(±)-Arterenol]	Sigma-Aldrich	A0937
L-Ascorbic Acid, Sodium Salt	Sigma-Aldrich	A7506
L-Glutamine, 200 mM	Sigma-Aldrich	G7513
Trypsin-EDTA (0.05%trypsin in 0.02% EDTA-Na)	Sigma-Aldrich	T3924
Trypsin Inhibitor Type I-S, Soybean	Sigma-Aldrich	T6522
Dulbecco's PBS (Ca^{2+}-free and Mg^{2+}-free)	Sigma-Aldrich	D8537
Fibronectin (1 mg/ml)	Sigma-Aldrich	F1141
Gelatin from bovine skin**	Sigma-Aldrich	G9391
Distilled Water, cell culture grade	Sigma-Aldrich	W3500
Cryovials, 2 ml round bottom	Corning	430289
Sterile Acrodisc syringe filters, 0.2µm	Gelman Sciences	4192
Freezing container	Nalgene	7-5100-0001

*If you have any problems obtaining this batch of serum or Claycomb Medium, please refer to **Reservation # 21864161**

or contact: Barbara Mullenschlader or SAFC Technical Services, Sigma-Aldrich, Inc.

**OR Bacto© Gelatin Fisher Scientific DF0143-17-9

Research Sales Services 314-286-8320 or Toll Free 800-220-0195

E-mail: Barb.Mullenschlader@sial.com or technicalservices@sial.com

This FBS has been pre-tested by the Claycomb lab for use with HL-1 cells and IT IS AN ABSOLUTE REQUIREMENT THAT THIS PARTICULAR BATCH OF SERUM BE USED WHEN CULTURING THESE CELLS. THE CELLS WILL NOT MAINTAIN THE CONTRACTING PHENOTYPE IF THIS FBS IS NOT USED. IT IS ONE OF THE MOST IMPORTANT REAGENTS.

Characteristics

Appearance–Clear orange-red solution

Endotoxin–Refer to Certificate of Analysis

Osmolality (as supplied)–Refer to Certificate of Analysis

pH (as supplied)–7.1 - 7.4

Sterility–No microbial growth detected

Reference

1. Claycomb, W., et al., (1998). Proc. Natl. Acad. Sci., 95:2979.

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