

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 is 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 cardiac therapeutic 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 4 mM L-glutamine, will maintain the HL-1 cell line and the mature cardiomyocyte behavior. While observing the HL-1 cells under light microscopy, individual and groups of cells can be observed contracting, becoming more frequent as the cardiomyocytes reach confluency.

Formulation

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

Precautions

Use aseptic 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.

Preparation Instructions

Supplement the medium with sterile 100 µM norepinephrine bitartrate (10 mL/L of a 10 mM norepinephrine stock solution), 4 mM L-glutamine (Catalog No. 59202C) and 10% FBS prior to use. Storage conditions and shelf life of the supplemented product will be affected by the nature of the supplements. Sterile serum should not be refiltered before or after being added to sterile medium because the growth promotion capacity may be reduced upon refiltration.

Methods for Use

For successful results, care must be taken when subculturing cells. Claycomb Medium is designed for use in a 5% CO₂ humid environment. HL-1 cells require higher than normal cell densities for optimal growth and behavior. In addition, HL-1 cells require a substrate that provides adequate anchorage during contraction and norepinephrine to maintain contractile activity.

Preparation of 10 mM Norepinephrine Stock Solution

1. Dissolve 1.92 mg of L-ascorbic acid 2-phosphate sesquimagnesium salt (CAS # 84309-23-9) in 25 mL of cell culture grade water (Catalog No. 59900C). This is a 0.3 mM L-ascorbic acid solution.
2. In a chemical safety fume hood, measure and add 33.73 mg norepinephrine bitartrate (CAS # 69815-49-2) to 10 mL of the 0.3 mM L-ascorbic acid solution. This is a 10 mM norepinephrine stock solution.
3. Sterile filter the norepinephrine stock solution with a 0.2 µm cellulose acetate filter.

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4. Use immediately (10 mL of the 10 mM norepinephrine stock solution per liter of Claycomb Medium) or aliquot into working volumes and store at -20 C for up to 2 months.

Substrate Coating

1. Prepare a 0.02% gelatin solution with cell culture grade water and sterile filter through a 0.2 µm low protein-binding filter.
2. Aseptically add 25 µg/mL fibronectin to the 0.02% gelatin solution.
3. Coat the culture flask with 2 mL/25 cm² surface area.
4. Seal and incubate the flask overnight at 37 C ± 1 C.
5. Transfer the flask to -10 to -40 C for storage up to 1 month.
6. Rinse the flask with sterile Dulbecco's Phosphate Buffered Saline (DPBS) (Catalog. No. 59321C) prior to use.

Cryopreservation

Freezing:

1. Choose cultures that are in logarithmic growth and of high viability.
2. Prepare a freezing medium consisting of 95% cold (4 to 8 C) FBS and 5% dimethyl sulfoxide (DMSO).
3. Using standard trypsinization methods, collect and centrifuge the cells at 250 g for 5 minutes. Remove the supernatant from the tube without disturbing the cell pellet.
4. Resuspend the cells in the freezing medium at a concentration of > 1 x 10⁷ cells/mL.
5. Aliquot 1 - 2 mL of this cell suspension into sterile cryovials.
6. Freeze the cells at a rate of 1 C/min.
7. For long-term storage, transfer the vials to a liquid nitrogen dewar.

Thawing:

1. Rapidly thaw a vial of cells in a 37 C ± 1 C water bath.
2. Dilute the cell suspension in 10 mL of supplemented Claycomb Medium and perform a viable cell count.
3. Using low-speed centrifugation, pellet the cells, and carefully decant the supernatant without disturbing the cell pellet.
4. Resuspend the cell pellet in freshly supplemented Claycomb Medium and seed into a sterile gelatin/fibronectin coated culture flask at 0.64-1.2 x10⁵/cm². Incubate in a humid environment at 37 C ± 1 C and 5% CO₂.
5. Replace the medium with freshly supplemented Claycomb Medium every 24 - 48 hours.
6. When the culture has reached a high confluency (typically 3 - 4 days), passage the culture using standard subculturing techniques.

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

References

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

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