Chondrogenic Differentiation Media

CHO.D.Media-450

Media Usage Protocol:

Chondrogenic Differentiation Media is designed to be used with Human Adipose Derived Mesenchymal Stem Cells or Human Bone Marrow Mesenchymal Stem Cells, both of which are available separately. When used as directed, this media will support differentiation of these stem cells into cartilage producing cells. The following is the recommended protocol for the usage of this media. Chondrogenic differentiation must be done in pellet culture and takes approximately 28 days.

Note: Once complete media has been formulated, it should be stored at 4°C. Avoid extended exposure of the media to room or higher temperatures. Media should be equilibrated in a water bath set at 37°C before adding to any cell culture. Chondrogenic Differentiation Media is serum free. Please do not add serum to the media.

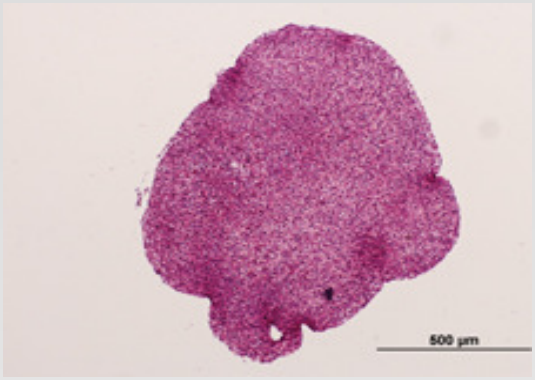

Figure 1: The figure shows a cartilage cell pellet derived after 28 days of differentiation of bone marrow derived mesenchymal stem cells using CET's Chondrogenic Differentiation Media. The cell was sectioned in a Paraffin block and stained with Safranin O.

Note: Antibiotics/ antimycotics should not be used as an alternative to proper aseptic technique.

Additional Reagents Needed

1. Penicillin/Streptomycin/Amphotericin B solution, 100X or Penicillin/Streptomycin solution, 100X. These solutions should be portioned in 5 mL aliquots, stored at -20°C and never freeze/thawed. Although antimycotics are not absolutely necessary, CET highly recommends their usage for long term cell culture.
2. A monolayer of actively growing adipose derived mesenchymal stem cells or bone marrow derived mesenchymal stem cells.

Formulating Complete Chondrogenic Differentiation Media

1. Add 5 mL of the antibiotic/antimycotic solution to the base Chondrogenic Differentiation Media.
2. Cap the bottle containing the now complete media and gently swirl a few times. The complete media is now ready to use.

Preparing Adipose or Bone Marrow Derived Mesenchymal Stem Cells

1. In a laminar flow hood, pipette spent complete Mesenchymal Stem Cell/Unrestricted Somatic Stem Cell Expansion Media from the cell monolayer and discard.
2. Wash the monolayer with Dulbecco's Phosphate Buffered Saline (DPBS). Use 10 mL/T-75 flask. Rock the flask gently, then remove the DPBS and discard.
3. Add 0.25% Trypsin/EDTA solution at 5 mL/T-75 flask. Rock the flask to spread the trypsin across the entire monolayer. Incubate at 37°C until the cells begin to detach. This should take approximately 5 minutes but no more than 15 minutes. Care must be taken that the cells are not forced to detach prematurely, as this may result in clumping.
4. Inactivate the trypsin by adding at least an equal volume of complete Mesenchymal Stem Cell/Unrestricted Somatic Stem Cell Expansion Media. Pipette the cells up and down to further separate into a single cell suspension.
5. Resuspend the cells in a 15 mL conical tube and centrifuge at 200 x g for 10 minutes. Carefully remove supernatant.

Cellular Engineering Technologies (CET) Inc.
2500 Crosspark Dr., Suite E232 Coralville, IA 52241
Phone: (319) 665-3000 Fax: (319) 665-3003

Chondrogenic Differentiation

1. Resuspend the cells in 10 mL complete Chondrogenic Differentiation Media. Remove a small sample for counting.
2. Count the cells with a hematocytometer or cell counter and calculate desired number. $1x10^6$ cells per pellet is recommended.
3. Centrifuge cells at 200 x g for 10 minutes to pellet cells in a 15 mL conical tube. Gently remove the supernatant without disturbing the pellet.
4. Carefully add 5 mL complete Chondrogenic Differentiation Media.
5. Loosen the cap of the conical tube with the pellet and incubate at 37°C, 5% CO_2.
6. Every three days, remove all media and replace with fresh, complete, Chondrogenic Differentiation Media. Be careful to never disturb the pellet. Chondrogenesis takes 28 days on average.

Key References:

1. Arthritis Res Ther. 2007;9(3):R55.
Hypoxic conditions increase hypoxia-inducible transcription factor 2alpha and enhance chondrogenesis in stem cells from the infrapatellar fat pad of osteoarthritis patients.
Khan WS, Adesida AB, Hardingham TE.

2. Stem Cells. 2007 Mar;25(3):750-60. Epub 2006 Nov 9.
Identification of common pathways mediating differentiation of bone marrow- and adipose tissue-derived human mesenchymal stem cells into three mesenchymal lineages.
Liu TM, Martina M, Hutmacher DW, Hui JH, Lee EH, Lim B.

Certificate of Analysis

All hematopoietic, mesenchymal and multipotent stem cells are evaluated by flow cytometry for specific stem cell markers. All other cells are evaluated either by staining, method of isolation or traditional molecular biology techniques. Data is available upon request.

All growth and differentiation media are evaluated by conducting assays to make sure cells either grow or differentiate as indicated on the media label. Data is available upon request.

All cells are tested for HIV-1, HIV-2, Hepatitis B and Hepatitis C using sensitive PCR based assays. All cells test negative for these viruses. However, all human cells must be used in accordance with established laboratory safety procedures and only under the supervision of trained personnel.

Table 1: Preparation of 450 mL complete Chondrogenic Differentiation Media

Brand	Amount For 450 mL	CET Product	Catalog #
CET	450 mL	CET Chondrogenic Differentiation Media	CHO.D.Media-450

Store at 4°C.

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celleng-tech.com