Osteogenic Differentiation Media
OST.D.Media-450

Media Usage Protocol:

Osteogenic Differentiation Media is designed to be used with Human Adipose Derived Mesenchymal Stem Cells and Human Bone Marrow Mesenchymal Stem Cells, all of which are available separately. When used as directed, this media will support differentiation of these stem cells into osteogenic or bone producing cells. The following is the recommended protocol for the usage of this media.

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.

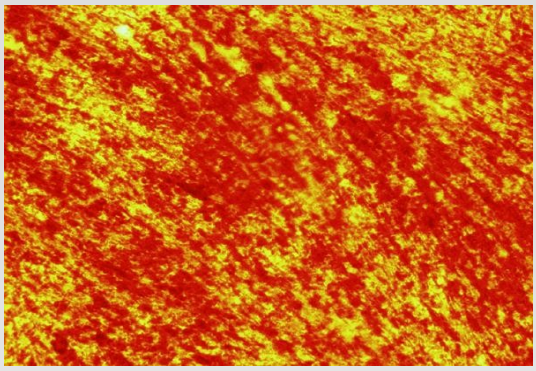

Figure 1: The figure shows osteogenic differentiation from adipose derived mesenchymal stem cells after 14 days in CET's Osteogenic Differentiation Media. Cells were stained with Alizarin Red S stain.

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

Additional Reagents Needed

1. Fetal Bovine Serum, High Grade or Characterized. Store in aliquots of 50mL at –20°C.
2. Penicillin/Streptomycin/Amphotericin B solution, 100X or Penicillin/Streptomycin solution, 100X. These solutions should be portioned in 5mL 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.
3. A monolayer of actively growing adipose derived mesenchymal stem cells or bone marrow derived mesenchymal stem cells.

Formulating Complete Osteogenic Differentiation Media

1. Defrost 50mL of fetal bovine serum and 5mL of antibiotic/antimycotic solution in a 37°C water bath until ice in the tubes is no longer visible.
2. Immediately disinfect the tubes and the bottle containing the base media with 70% isopropranol.
3. Working in a laminar flow hood, remove 5mL of the media from the bottle and discard. This and all other procedures must be done in a sterile manner.
4. Add 50mL of the fetal bovine serum to the base media.
5. Add 5mL of the antibiotic/antimycotic solution to the base media.
6. 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/Multipotent Unrestricted Somatic Stem Cell Expansion Media from the cell monolayer and discard.
2. Wash the monolayer with Dulbecco's Phosphate Buffered Saline (DPBS). Use 10mL/T-75 flask. Rock the flask gently, then remove the DPBS and discard.
3. Add 0.25% Trypsin/EDTA solution at 5mL/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. Centrifuge the cells at 200 x g for 10 minutes. Carefully remove supernatant.
6. Resuspend the cells in 10mL complete Mesenchymal Stem Cell/Unrestricted Somatic Stem Cell Expansion Media.
7. Plate the cells on a fresh tissue culture dish at 80% confluency using complete Mesenchymal Stem Cell/Unrestricted Somatic Stem Cell Expansion Media. Osteogenic differentiation prefers higher confluency of cells so make sure the tissue culture flask is atleast 80% confluent.
8. Let cells attach for 24 hours or until normal morphology is seen. Once the cells have attached and reached 80% confluency, withdraw the complete Mesenchymal Stem Cell/Unrestricted Somatic Stem Cell Expansion Media.
9. Rinse the monolayer twice with Dulbecco's Phosphate Buffered saline.

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Osteogenic Differentiation
1. Add complete Osteogenic Differentiation Media. For a T-75 flask, about 15mL is sufficient. Add more or less media depending on the size of the culture vessel.
2. Incubate the cells at 37°C, 5% CO_2, with humidity.
3. Every 72 hours withdraw and add new complete Osteogenic Differentiation Media. Osteogenic differentiation can be seen visually as a striation of cells or by calcium staining using Alizarin Red S. Osteogenic differentiation can take between 21 and 28 days.

Key References:
1. J Bone Miner Res. 2008 Dec 8.
miR-196a Regulates Proliferation and Osteogenic Differentiation in Mesenchymal Stem Cells Derived From Human Adipose Tissue.
Kim YJ, Bae SW, Yu SS, Bae YC, Jung JS.

2. Life Sci. 2008 Dec 19;83(25-26):851-8.
ICAT participates in proliferation and osteogenic differentiation of human adipose tissue-derived mesenchymal stem cell.
Kim YJ, Kim JT, Bae YC, Suh KT, Jung JS.

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 500 mL complete Osteogenic Differentiation Media

Brand	Amount For 500 mL	CET Product	Catalog #
CET	450 mL	CET Osteogenic Differentiation Media	OST.D.Media-450
Any	50 mL	Fetal Bovine Serum	Refer to Manufacturer's Catalog Number

Store at 4°C.

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