Mesenchymal Stem Cell Chondrogenic Differentiation Medium W/O inducer (MCDM)

Catalog Number: 7551 (500ml)

Product Description

Our chondrogenic differentiation medium without differentiation inducer has been specifically developed and optimized for *in vitro* mesenchymal stem cell chondrogenesis study. Mesenchymal stem cell Chondrogenic Differentiation medium (MCDM) is a sterile, liquid medium which contains essential and non-essential amino acids, vitamins, organic and inorganic compounds, hormones, growth factors, trace minerals. The medium is HEPES and bicarbonate buffered and has a pH of 7.4 when equilibrated in an incubator with an atmosphere of 5% CO_2/95% air.

Components

MCDM consists of 500 ml of basal medium, 5 ml of mesenchymal stem cell chondrogenic differentiation supplement (MCDS, Cat. No. 7582-5), 5 ml of penicillin/streptomycin solution (P/S, Cat. No. 0503-5).

Note: *To complete mensenchymal stem cell chondrogenic differentiation medium, our MCDM must be supplemented with adequate chondrogenic inducer, 10 ng/ml TGF-β3, by the customer.*

Product Use

MCDM is for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Store the basal medium at 4°C, the MCDS and the P/S solution at -20°C. Protect from light.

Shipping

Gel ice.

Prepare for use

Thaw MCDS and P/S solution at 37°C. Gently tilt the MCDS tube several times during thawing to help the contents dissolve. **Make sure the contents of the supplement are completely dissolved into solution before adding to the medium**. Rinse the bottle and tubes with 70% ethanol, and then wipe to remove excess. Remove the cap, being careful not to touch the interior threads with fingers. Add MCDS and P/S solution into basal medium in a sterile field, mix well and then the reconstituted medium is ready for use. Since several components of MCDM are light-labile, it is recommended that the medium not be exposed to light for lengthy periods of time. If the medium is warmed prior to use, do not exceed 37°C. When stored in the dark at 4°C, the reconstituted medium is stable for one month.

Caution: If handled improperly, some components of the medium may present a health hazard. Take appropriate precautions when handling it, including the wearing of protective clothing and eyewear. Dispose of properly.

Instruction for Chondrogenic Differentiation

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C water bath and return them to culture as quickly as possible with minimal handling!

Set up of Expansion Culture for Differentiation:

1. Primary Mesenchymal Stem Cells (MSCs) should be expanded with MSCM (cat # 7501) in T-25 or T-75 flasks, which have been coated with poly-l-lysine and placed for at least 1 hour in the 37°C incubator.
2. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture.
3. Change the medium every other day thereafter, until the culture is ready for subculture.
4. In general, human MSCs can be subcultured every 3 to 4 days.

Induction of Chondrogenic Differentiation:

1. Prepare complete chondrogenic differentiation medium: Thaw supplement MCDS and penicillin/streptomycin solution (P/S, Cat. No. 0503) at room temperature, or at 37°C water bath. Prepare as below.

Chondrogenic differentiation medium	Stock Conc.	Final Conc.	For 50 ml
MCDM (Cat. No. 7551)	1 X		49 ml
MCDS (Cat. No. 7582)	100 X		0.5 ml
P/S (Cat. No. 0503)	100 X		0.5 ml
**TGF-β3 (differentiation inducer)	10 ug/ml	10 ng/ml	50 ul

: TGF-β3 is not included in ScienCell chondrogenic differentiation medium. It needs to be provided by the customer.

2. Passaged MSCs are centrifuged at 500 g for 5 minutes.
3. Cells are resuspended at a density of 0.5 to 1 X 10^6 cells/ml in complete chondrogenic differentiation medium or mesenchymal stem cell medium (MSCM, Cat. No. 7501) as a negative control.
4. Transfer 1 ml of the cell suspension into 15 ml polypropylene centrifuge tube.
5. Centrifuge the cell suspension at 500 g for 5 minutes.

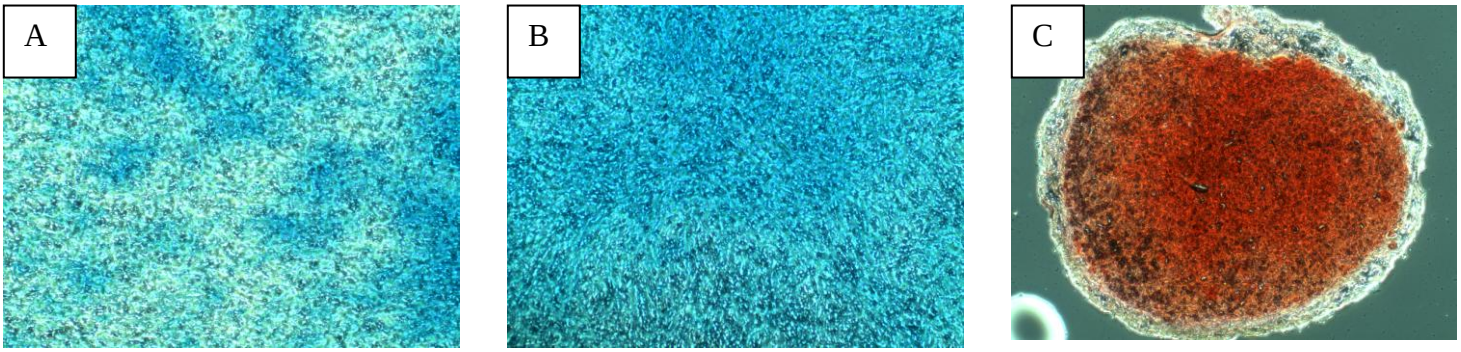
6. Place the polypropylene centrifuge tube containing cell pellet into incubator at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

   Note: The caps of the tubes should be loosened to allow air exchange.

7. Spheroids will form within 24 hr. The more cells you use, the bigger the spheroids.
8. Replace spent medium every third day. Be careful not to aspirate the spheroids.
9. After 4 weeks of culture, chondrogenic cell aggregates can be processed for Safranin-O or Alcian Blue staining, protein detection, gene expression or immunohistochemistry.

Safranin-O Stain Analysis:

1. The spheroids are fixed in 4% paraformaldehyde solution for 3 hours.
2. Deparaffinize and hydrate slides to distilled water.
   - 2.1 Deparaffinize in Xylene, 3 changes x 5 minutes.
   - 2.2 Hydrate in 100% Ethanol, 2 changes x 2 minutes.
   - 2.3 Hydrate in 95% Ethanol, 2 change x 2 minutes.
   - 2.4 Hydrate in 70% Ethanol, 1 change x 1 - 2 minutes.
   - 2.5 Hydrate in 50% Ethanol, 1 change x 15 minutes.
   - 2.6 Rinse in running tap water, 1 x 10 minutes.
3. Stain in 0.1% Safranin O solution for 5 minutes.
4. Dehydrate and clear with 95% ethyl alcohol, absolute ethyl alcohol, and xylene, using 2 changes each, 2 minutes each.
5. Mount using resinous medium.



Analysis of MSCs (Cat. No. 7500) cultured in MCDM (Cat. No. 7551) supplemented with 10 ng/ml TGF-β3 for (A) 2 weeks and (B) 4 weeks demonstrated differentiation of mesenchymal stem cell to chondrogenic lineage by Alcian Blue staining, or by (C) Safranin O staining of cross-section of cell spheroids.