HiChondroXL™ Chondrocyte Differentiation Supplement

Product Code: TCL169

Product Description:

HiChondroXL™ Chondrocyte Differentiation Supplement is optimized for chondrogenic differentiation of actively proliferating human mesenchymal stem cells *in vitro*.

Clonally expanded mesenchymal stem cells (MSCs) have ability to differentiate into three types of cells - adipocytes, osteocytes and chondrocytes. Differential potential of MSCs in these three cell types is considered as a reliable functional criterion to identify MSCs and distinguish them from preadipocytes, preosteocytes and prechondrocytes, each of which gives rise to only one cell type. Chondrogenesis *in vitro* follows a highly ordered and well characterized temporal sequence.

TCL169 is a proprietary supplement formulated to contain induction factors that induce chondrogenic differentiation of human mesenchymal stem cells into proteoglycan producing chondrocytes.

Materials required but not provided:

1. Growth medium
   a. HiMesoXL™ Mesenchymal Stem Cell Expansion Medium (AL512) OR
      Dulbecco's Modified Eagle Medium, High glucose (AL007A)
2. Media supplements
   a. Fetal Bovine Serum (FBS) (RM1112/ RM10432)
   b. Antibiotic-Antimycotic Solution 100X (A002)
3. Reagents for subculture
   a. Dulbecco's Phosphate Buffered Saline (DPBS) (TL1006)
   b. Trypsin/EDTA Solution 1X (TCL007)
   c. Soybean Trypsin Inhibitor Solution (TCL068)
   d. Trypan Blue 0.5% solution (TCL005)
4. Chondrocyte Staining
   a. Alcian blue OR
   b. Safranine (TC260) OR
   c. Toluidine blue O (TC257)
5. Consumables

General Guidelines:

Follow the below mentioned guidelines for optimal adipogenic differentiation.

Passage number

Use the cells with low passage number (less than 5 passages). Mesenchymal stem cells tend to lose their differentiation potential with increasing passage number. Use of cells with high passage number cells might lead to false-positive or false-negative results.

Passage timing

During regular maintenance, subculture the cells when they are 70 - 80% confluent. Do not allow them to reach 100% confluency as it results in loss of multipotency of the cells.

Cell dissociation

Avoid prolonged exposure of cells to trypsin during subculture. Prolonged exposure causes reduced viability and expansion capacity of cells. Monitor the trypsinization procedure carefully and neutralize it immediately upon dissociation.

Assay controls

Use appropriate assay controls for comparing differentiated cells with undifferentiated cells.

Culture conditions

Chondrocyte differentiation requires cells to be grown in a three-dimensional aggregate culture system. Easy and convenient method to grow the cells in this manner is to culture them in polypropylene centrifuge tubes. Cells form aggregates in tubes without adhering to the tube surface. Use of polystyrene tubes is not recommended because cells tend attach to the polystyrene surface and fail to form aggregates.

Other methods to culture the cells in three-dimensional system include use of U-bottom non-treated culture well plate, or use of calcium alginate encapsulation technique.

Directions:

Users are advised to review entire procedure before starting the assay.

Preparation of mesenchymal stem cells for chondrogenic differentiation

1. Maintain the mesenchymal cells in HiMesoXL™ Mesenchymal Stem Cell Expansion Medium (AL512) supplemented with 10% FBS (RM1112/ RM10432).
2. Observe the cells every day under the microscope for morphology and confluency.
3. Replace the medium if required.
4. When the cells are 70% confluent, they are ready for subculturing.
5. Aseptically remove spent medium and wash the monolayer gently using appropriate volume of DPBS.
   Note: *Rock the flask gently. Take care not to disturb the monolayer.*
6. Remove and discard DPBS.
7. Add trypsin in an amount sufficient to cover the monolayer.
8. Incubate at 37°C in a 5% CO_2 humidified incubator for 3 - 5 minutes.
9. Carefully monitor the cell dissociation.
10. As soon as the cells dissociate from the surface, neutralize the action of trypsin by adding complete medium or Soybean Trypsin Inhibitor solution (TCL068).
    Note: *Here, complete medium refers to AL007A with 10% FBS or AL512 with 10% FBS*
11. Aseptically collect the cells in a sterile centrifuge tube and centrifuge at 500 - 600rpm for 5 - 7 minutes to remove the traces of trypsin.
12. Discard the supernatant and resuspend the pellet in complete medium.
13. Determine cell density and cell viability using trypan blue and hemocytometer.
14. Prepare the cell suspension in AL007A or AL512 supplemented with 10% FBS and adjust the cell density to 1 X 10^6 cells/ml.

Preparation of complete chondrogenic differentiation medium

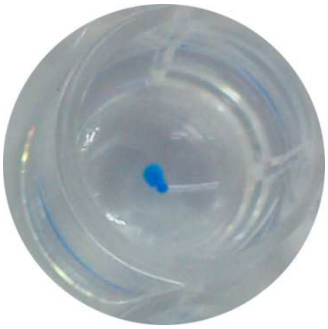
1. Thaw HiChondroXL™ chondrogenic differentiation supplement (TCL169) at 2-8°C overnight.
   Note: *Precipitates in the supplement after thawing are normal. Precipitation will not affect performance of the medium*.
2. Disinfect the external surface of the bottles by spraying with isopropyl alcohol before placing in a bisafety hood.
3. Transfer the entire content of TCL169 to 100 ml of basal media under aseptic condition.
   Note: *If desired, 1ml antibiotic-antimycotic solution (A002) can be added to 100ml of complete medium.*
4. Tightly cap the bottle and swirl gently to ensure proper mixing.
   Note: *Do not mix vigorously. Doing so will cause formation of foam.*
5. Store the complete differentiation medium at 2 - 8°C until use.

Procedure for induction of chondrogenic differentiation

1. Aseptically add 1ml of cell suspension (1 X 10^6 cells/ml density) in a sterile polypropylene 15ml centrifuge tube.
2. Centrifuge at 1000rpm for 10 minutes.
3. Incubate the tube at 37°C in a 5% CO_2 humidified incubator for 48 hours without disturbing the pellet.
   Note: *Do not cap the tubes tightly. Keep them loose to allow gas exchange*.
4. After 48 hours remove the tubes from incubator.
5. Discard the supernatant without disturbing the cell pellet.
6. Add 1ml of complete chondrocyte differentiation medium to the tube. Resuspend the cell pellet by gentle pipetting.
7. Centrifuge at 1000rpm for 10 minutes.
8. Incubate the tube at 37°C in a 5% CO_2 humidified incubator for 48 hours without disturbing the pellet. This is considered as Day 1 of chondrogenic differentiation.
9. Repeat steps 4 to 8 after every 48 hours till 18 - 21 days of chondrogenic differentiation.
10. Cells aggregate and form spheroids that can be seen with naked eyes.
11. Stain the spheroids with Alcian blue or Toluidine blue or safranine (Refer Figure 2 and 3).

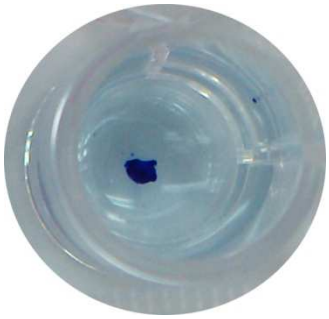
Observation:

Figure 1



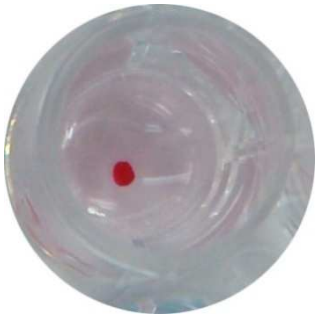
Spheroid of chondrocytes stained with Alcian blue

Figure 2



Spheroid of chondrocytes stained with Toluidine blue

Figure 3



Spheroid of chondrocytes stained with Safranine

Quality control:

Appearance

Pale yellow coloured clear solution

Sterility

No bacterial or fungal growth is observed after 14 days of incubation, as per USP specification.

Cultural Response

Chondrogenic differentiation potential of the medium is assessed by differentiating mesenchymal stem cells for 18 - 21 days in the medium and analyzing them qualitatively for presence of proteoglycans by Alcian blue staining method.

Storage and shelf life:

Shelf life is 12 months at -20^{0}C
Shelf life of complete medium after reconstitution with TCL169 is 6 weeks at 2-8^{0}C
Note: *Freezing of the basal medium and complete medium is not recommended. Avoid repeated freezing and thawing of the growth supplement.*

In vitro diagnostic medical device

CE Marking

Consult instructions for use

Do not use if package is damaged

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