HiAdipoXL™ Adipocyte Differentiation Supplement

Product Code: TCL167

Product Description:

HiAdipoXL™ Adipocyte Differentiation Supplement is optimized for adipogenic 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. Adipogenesis *in vitro* follows a highly ordered and well characterized temporal sequence.

TCL167 is a proprietary supplement formulated to contain induction factors that induce adipogenic differentiation of human mesenchymal stem cells.

Materials required but not provided:

1. Growth medium
   a. HiMesoXL™ Mesenchymal Stem Cell Expansion Medium (AL512) OR
      DMEM, High glucose (AL007A)

2. Media supplements
   a. Fetal Bovine Serum (FBS) (RM1112/ RM10432)
   b. Antibiotic-Antimycotic Solution 100X (A002)

3. Reagents
   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. Staining
   EZStain™ Adipocyte Staining Kit (CCK013)

5. Consumables

General Guidelines:

Follow below mentioned guidelines for optimal adipogenic differentiation.

Passage number

Use 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 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.

Directions:

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

Preparation of mesenchymal stem cells for adipogenic differentiation

1. Maintain the mesenchymal stem 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. When the cells are 70% confluent, they are ready for subculturing.
4. 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. Discard the DPBS.*
5. Add trypsin in an amount sufficient to cover the monolayer.
6. Incubate at 37°C for 3 - 5 minutes in a 5% CO_2 humidified incubator.
7. As soon as cells dissociate from the surface, neutralize the action of trypsin by adding equal amount of complete medium or Soybean Trypsin Inhibitor (TCL068).
   Note: *Here, complete medium refers to AL007A with 10% FBS or AL512 with 10% FBS.*
8. Aseptically collect the cells in a sterile centrifuge tube and centrifuge at 500 - 600rpm for 5 - 7 minutes to remove the traces of trypsin.
9. Discard the supernatant and resuspend the pellet in complete medium.
10. Determine cell density and cell viability using trypan blue and hemocytometer.
11. Prepare the cell suspension in AL007A or AL512 supplemented with 10% FBS and seed with 5000 cells/cm^2 density in a desired culture vessel.
    Note: *Refer Table 1 for recommended culture volumes for different culture vessels.*

Table 1: Suggested working volumes of media for different culture vessels

Culture vessel	Medium volume	No. of wells differentiated using 100ml AL521
48-well plate	500µl	200
24-well plate	1ml	100
12-well plate	1ml	100
6-well plate	2ml	50

15. Gently rock the plate back and forth and side to side to distribute the cells evenly before incubation. Do not swirl.
16. Incubate the plate at 37°C in a 5% CO_2 humidified incubator until the cells are 70% confluent (approximately 48-72 hours).
17. Once the cells reach 70% confluence, they are ready for osteogenic differentiation.

Preparation of complete adipogenesis differentiation medium

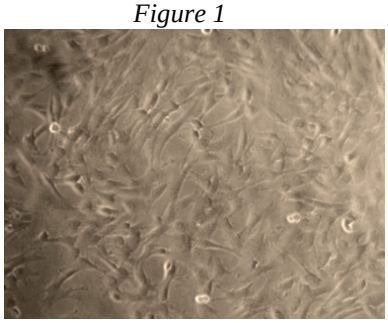
1. Thaw HiAdipoXLTM Adipogenic Differentiation supplement (TCL167) at 2-8°C overnight.
   Note: Precipitates in this supplement after thawing are normal. Precipitation will not affect performance of the medium.
2. Disinfect the external surface of the bottle by spraying with isopropyl alcohol before placing in a biosafety hood.
3. Transfer the entire content of TCL167 to 100 ml of basal media under aseptic conditions.
   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 adipogenic differentiation

1. Take out the plate from incubator and aseptically remove the spent medium. Add appropriate volume of complete differentiation medium. (Refer table 1 for recommended volumes of medium).
   Note: *This medium change is considered as Day 1 of adipogenic differentiation.*
2. Observe the cells microscopically and replace the medium with fresh complete differentiation medium after every 48 - 72 hours.
   Note: *Gently add and remove the medium from the culture vessel to avoid detachment of cells from vessel surface. Add the medium along the side of culture wells. Do not tilt the culture plate while aspirating the medium. Do not expose the monolayer to air as it will cause bursting of lipid vesicles formed in differentiating cells.*
3. Continue this procedure for next 18 to 21 days.
4. Lipid vesicles formed in the cells can be observed microscopically as intracellular oil droplets. (Refer Figure 1).
5. Stain the lipid droplets using EZStain™ Adipocyte Staining Kit (CCK013). (Refer Figure 2).

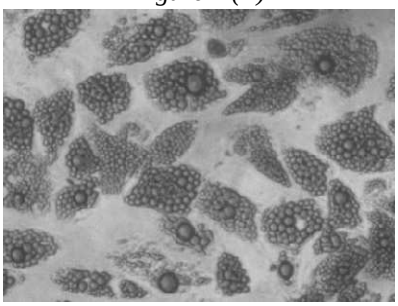
Observation:

Figure 1



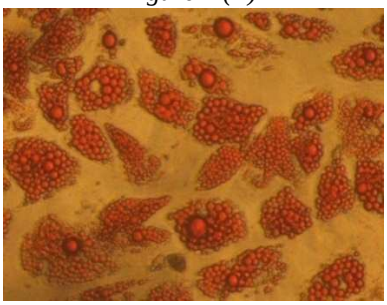
Undifferentiated Human Adult Mesenchymal Stem cells unstained with Oil Red O (40X)

Figure 2(A)



Human adult mesenchymal stem cells differentiated into adipocytes before staining with Oil Red O (40X)

Figure 2(B)



Human adult mesenchymal stem cells differentiated into adipocytes after staining with oil red O
Lipid droplets stained bright red/orange with Oil-Red-O staining solution (40X)

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

Adipogenic differentiation potential of the medium is ssessed by differentiating mesenchymal stem cells for 18-21 days in the medium and analyzing them quantitatively for presence of oil droplets by oil-red-o staining method.

Storage and Shelf Life:

Shelf life is 12 months at -20^{0}C
Shelf life of complete medium after reconstitution with TCL167 is 6 weeks at 2-8^{o}C

Note: *Freezing of the basal medium and complete medium is not recommended. Avoid repeated freezing and thawing of the growth supplement.*

Related products:

EZStain™ Adipocyte Staining Kit
Code: CCK013
EZDiff™ 3T3 L1 Differentiation Kit
Code: CCK011

IVD	In vitro diagnostic medical device
CE	CE Marking
	Consult instructions for use
	Do not use if package is damaged
	HiMedia Laboratories Pvt. Limited, B /4-6 , MIDC, Dindori, Nashik MH www.himedialabs.com
EC REP	CE Partner 4U ,Esdoornlaa 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu

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