

HiOsteoXL™ Osteocyte Differentiation Medium

Product Code: AL522

Product Description:

HiOsteoXL™ Osteocyte Differentiation Medium is optimized for osteogenic 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, chondrocytes and osteocytes. Differential potential of MSCs in these three cell types is considered as a reliable functional criterion to identify MSCs and distinguish them from preadipocytes, prechondrocytes and preosteocytes, each of which gives rise to only one cell type. Osteogenesis *in vitro* follows a highly ordered and well characterized temporal sequence.

AL522 is HiOsteoXL™ Osteocyte Differentiation Medium consisting of two parts -

Part A: HiOsteoXL™ Basal Medium

It is a basal medium containing organic and inorganic salts, amino acids, vitamins and sodium bicarbonate. It is devoid of antibiotics and antimycotics.

Part B: HiOsteoXL™ Osteogenic Differentiation Supplement

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

HiOsteoXL™ Osteogenic Differentiation Medium contains phenol red. Use of phenol red containing medium may enhance and accelerate rate of calcium deposition during osteogenic differentiation.

Materials required but not provided:

1. Expansion 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 and medium 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. Osteocyte staining
 - a. Alizarin Red S (TC255)
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.

Directions:

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

Preparation of mesenchymal stem cells for osteogenic differentiation

- Expand the mesenchymal cells in HiMesoXL™ Mesenchymal Stem Cell Expansion Medium (AL512) with 10% FBS.
- Observe the cells every day under the microscope for morphology and confluency.
- Replace the medium if required.
- When the cells are 70% confluent, they are ready for subculturing.
- 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.
- Remove and discard DPBS.
- Add trypsin in an amount sufficient to cover the monolayer.
- Incubate at 37°C in a 5% CO₂ humidified incubator.
- Carefully monitor the cell dissociation.
- 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.
- Aseptically collect the cells in a sterile centrifuge tube and centrifuge at 500 - 600rpm for 5 - 7 minutes to remove the traces of trypsin.
- Discard the supernatant and resuspend the pellet in complete medium.
- Determine cell density and cell viability using trypan blue and hemocytometer.
- Prepare the cell suspension in AL007A or AL512 with 10% FBS and seed with 5000 cells/cm² 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 per well	No. of wells differentiated using 100ml AL522
48-well plate	500µl	200
24-well plate	1ml	100
12-well plate	1ml	100
6-well plate	2ml	50

- Gently rock the plate back and forth and side to side to distribute the cells evenly before incubation. Do not swirl.
- Incubate the plate at 37°C in a 5% CO₂ humidified incubator until the cells are 70% confluent (approximately 48-72 hours).

- Once the cells reach 70% confluence, they are ready for osteogenic differentiation.

Preparation of complete osteogenic differentiation medium

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

Procedure for induction of adipogenic differentiation

- Take out the plate from incubator and observe microscopically for confluence and morphology of cells. If the cells exhibit healthy morphology and are 70% confluent, use the plate for osteogenic differentiation.
- Aseptically remove half the volume of spent medium from each well and add appropriate volume of fresh medium.
Note: For e.g., in case of a 6-well plate, total culture volume is 2ml. Remove 1ml spent medium from each well and then add 2ml of fresh medium.
- Refer table 2 for recommended volumes of medium to be added depending on culture vessel.
- Incubate the plate at 37°C in a 5% CO₂ humidified incubator.

Table 2: Suggested volumes of media for medium change for different culture vessels

Culture vessel	Volume of medium per well at the time of seeding	Volume of fresh medium to be added per well
48-well plate	500µl	500µl
24-well plate	1ml	1ml
12-well plate	1ml	1ml
6-well plate	2ml	2ml

Note: Calcium deposits formed by osteocytes are extracellular. Removal of entire spent medium may lead to loss of calcium deposits. Do not tilt the plate while changing the medium. Tilting may also lead to exposure of cell monolayer to air and loss of calcium deposits.

During differentiation procedure, cells tend to peel off from the vessel surface. Gently add and remove the medium from culture vessel to avoid detachment of cells from vessel surface. Add the medium along the side of culture wells.

This medium change is considered as Day 1 of differentiation.

5. Observe the cells microscopically and replace the medium with fresh complete differentiation medium after every 48 - 72 hours. Osteoblasts appear tightly packed and linear in shape.

Note: At this step, volume of the fresh medium to be removed and added will remain the same.

For. e.g. in case of a 6-well plate, remove 2ml of spent medium and add 2ml of fresh medium.

6. Incubate the plate at 37°C in a 5% CO₂ humidified incubator.
7. Continue steps 5 and 6 for next 18 to 21 days.
8. Differentiation of mesenchymal stem cells to osteocytes can be detected by several methods such as surface marker analysis by immunostaining, gene expression analysis, protein detection, Alizarin Red S staining for calcium deposits and alkaline phosphatase detection.

Suggested staining methods for detection of osteogenic differentiation:

Alizarin Red S Staining:

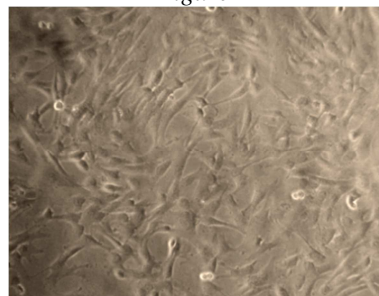
Undifferentiated mesenchymal stem cells have no extracellular calcium deposits whereas differentiated osteoblasts exhibit large amount of extracellular calcium deposits *in vitro*. Calcium deposits are therefore an indication of successful differentiation of MSCs into osteoblasts. Calcium deposits can be specifically stained with Alizarin Red S. Osteoblasts appear bright orange-red in colour. Undifferentiated MSCs do not exhibit the colour.

Alkaline Phosphatase (AP) Staining:

Undifferentiated mesenchymal stem cells exhibit weak alkaline phosphatase activity whereas differentiated osteoblasts show very high alkaline phosphatase activity. AP activity is therefore an indication of successful differentiation of MSCs into osteoblasts.

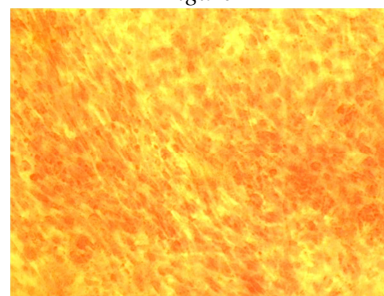
Observation:

Figure 1



Undifferentiated Human Adult Mesenchymal Stem cells (40X)

Figure 2



Calcium deposits in mesenchymal stem cells differentiated into osteocytes stained red in colour by Alizarin Red S (40X)

Quality control:

Appearance

Part A: Orangish red coloured clear solution
Part B: Pale yellow coloured clear solution

pH

7.00 -7.60

Osmolality in mOsm/Kg H₂O

320.00 - 360.00

Sterility

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

Cultural Response

Osteogenic 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 calcium deposits by Alizarin red S and alkaline phosphatase staining method.

Storage and shelf life:

Store HiOsteoXL™ Basal Medium (Part A) at 2-8°C away from bright light.

Store HiOsteoXL™ Osteogenic Differentiation Supplement (Part B) at -20°C.

Use before expiry date given on the product label.

Shelf life of the complete medium is 6 weeks at 2-8°C.

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

Related products:

HiAdipoXL™ Adipocyte Differentiation Medium

Code: AL521

HiOsteoXL™ Chondrocyte Differentiation Medium

Code: AL523

Alizarin Red S

Code: TC255

Revision: 1 / 2012

Disclaimer:

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