Mesenchymal Stem Cell Osteogenic Differentiation Kit

Cat # 5011-024-K

Reagents for differentiating Mesenchymal Stem Cells into Osteoblasts

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Table of Contents

Introduction

Mesenchymal Stem Cells can differentiate into osteogenic cells[1,2] that will deposit and mineralize extracellular matrix proteins,[2,3] which is required for the formation of new bone. Measuring the amount of mineralized matrix is an established method for evaluating osteogenic differentiation.[2]

Mesenchymal stem cells are a self-renewing population of multipotent cells, present in the bone marrow and many other adult tissues, which can differentiate into multiple lineage-specific cells that form bone, fat, cartilage, muscle and tendon.[1-8] The differentiation of mesenchymal stem cells into osteogenic cells can be a useful tool for understanding mechanisms involved in bone formation and for identifying potential treatments for osteoporosis, bone repair and genetic disorders such as osteogenesis imperfecta.[3]

Trevigen's **Mesenchymal Stem Cell Osteogenic Differentiation Kit** contains reagents optimized to direct rat mesenchymal stem cells (RMSC) (cat# 5000-001-01) or human mesenchymal stem cells (HMSC) grown on Cultrex® Rat Collagen I[9] (cat# 3440-100-01) to undergo osteogenic differentiation in a defined growth medium supplemented with ascorbic acid, β-glycerol phosphate, dexamethasone and within 14 days. Osteogenic differentiation is detected by staining with Alizarin Red S[8]. Alizarin red will stain the calcium deposits that occur when differentiated mesenchymal stem cells mineralize the extracellular matrix.[8]

II. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.
2. The physical, chemical, and toxicological properties of these products may not yet have been fully investigated; therefore, Trevigen recommends the use of gloves, lab coats, and eye protection while using these chemical reagents. Trevigen assumes no liability for damage resulting from handling or contact with these products.

III. Materials Supplied

Component[1]	Quantity	Storage	Catalog#
Dexamethasone	12 µl	-20°C*	5010-024-01
Ascorbic Acid	1.2 ml	4°C	5011-024-01
β-glycerol Phosphate	1.2 ml	4°C	5011-024-02
Cultrex® Rat Collagen I[2]	500 µl	4°C	3440-001-01
Alizarin Red S[3]	25 ml	RT in dark	5011-024-03
Stain Solubilization Solution[3]	35 ml	RT	5011-024-04

1: Reagents are sufficient to differentiate one 24 well plate for up to 21 days
2: Sufficient rat tail collagen type I is provided to coat two 24 well plates
3: Enough reagent is provided to test two 24 well plates.
*Separate from shipping box and store at specified temperature

IV. Materials/Equipment Required But Not Supplied

Equipment

1. 1 - 20 µl, 20 - 200 µl, and 200 - 1000 µl pipettors
2. Laminar flow hood or clean room
3. 37˚C CO_2 incubator
4. 37˚C Water Bath
5. Hemocytometer or other means to count cells
6. Inverted standard or phase microscope
7. pipette aid
8. Liquid Nitrogen Storage
9. Low speed swinging bucket centrifuge and tubes for cell harvesting
10. Plate Reader

Reagents

1. Mesenchymal Stem Cells (Trevigen's RMSC 5000-001-01 or human equivalent)
2. Qualified MSC Medium (for RMSC: cat# 5000-500-03) or equivalent
3. Cell Harvesting Reagent, trypsin, dispase, etc.
4. Qualified MSC FBS (for RMSC: cat# 5000-050-02)
5. Antibiotic Supplement for Medium (optional)
6. Sterile PBS (Mg^{2+}, Ca^{2+} free) or HBSS
7. Trypan blue or equivalent viability stain
8. DMSO
9. 70% Ethanol
10. 10% Formalin

Disposables

1. Cell culture flask, 25 cm^2, 75 cm^2, or 185 cm^2
2. 24 well Tissue Culture Plates
3. 15 ml tubes
4. 0.22 µm Filter Unit (optional)
5. 60 ml Syringe
6. 1 - 200 µl and 200 - 1000 µl pipette tips
7. 1, 5 and 10 ml serological pipettes
8. gloves
9. Clear 96-well plate
10. Cryovials

V. Reagent Preparation

These procedures should be performed in a biological hood utilizing aseptic technique to prevent contamination.

1. Mesenchymal Complete Growth Medium

For 250 ml of Medium:

Qualified MSC Medium:	225 ml
Qualified FBS:	25 ml

Optional: media can be filter sterilized before use

Store media @ 4°C for one month

Ensure media are at room temperature or 37°C prior to use

2. Mesenchymal Freeze Medium

For 10 ml of Medium:

Qualified MSC Medium:	4 ml
Qualified FBS:	4 ml
DMSO:	2 ml

Mix 1:1 with Growth Medium before use

3. Osteogenic Differentiation Medium*

For 13 ml of Medium

Mesenchymal Growth Medium (From V.I):	12.96 ml
Dexamethasone	1.3 µl
Ascorbic Acid	130 µl
β-Glycerol Phosphate	130 µl

Invert several times to mix.

*Make up Fresh Prior to use

Ensure medium is at 37°C prior to use, addition of cold media can induce "peeling" of cells.

4. Collagen Solution for Coating

To coat one 24 well plate, add 250 µl Cultrex® Collagen I to 12.5 ml of Qualified RMSC Medium (adjust volume as needed accordingly to number of wells)

VI: Protocol

These procedures should be performed in a biological hood utilizing aseptic technique to prevent contamination.

A. Thawing Mesenchymal Stem Cells:

1. Prepare Mesenchymal Complete Growth Medium (Section V.1):
2. Prewarm Complete Growth Medium to 37 °C by placing in 37°C H_2O bath or in Tissue Culture Incubator
3. Immediately before use, remove vial of cryopreserved mesenchymal stem cells (MSC) (e.g. Rat MSC cat# 5000-001-01) from liquid nitrogen freezer.
4. Thaw frozen RMSC quickly in a 37°C H_2O bath.
   a. Ensure cells are completely thawed before proceeding
   b. Do not leave cells at 37°C for fast thawing
5. Spray down bottle containing Complete Growth Medium and ampoule containing cells with 70% EtOH before placing in Tissue Culture Hood
6. Aseptically, transfer the thawed cells to a 15 ml conical tube with a 5 ml pipette

7. Add 1 ml of warm Complete Growth Medium to ampoule using a 5 ml pipette, dropwise with gentle swirling of the cells and transfer to a 15 ml conical tube dropwise, gently swirling to mix between drops
8. Add 1 ml of warm Complete Growth Medium to 15 ml conical tube containing cells, gently swirling to mix between drops
   Note: Total Volume should be about 3 ml
9. Centrifuge 15 ml conical tube at 250 x g for 5 minutes at room temperature
10. Remove supernatant gently to avoid disturbing cell pellet
11. Resuspend cell pellet in 1 ml of fresh Complete Growth Medium
12. Count cells on hemocytometer (per standard protocol)
13. For Trevigen's RMSC (cat# 5000-001-01): Plate cells at a density of $5.4x10^3$ cell/cm^2 in a T-75 tissue culture treated flask ($4.05x10^5$ cells) in a total volume of 12-15 ml Complete Growth Medium
    Notes: Recommend Corning Tissue Culture Treated Plastic. One vial is sufficient to seed two T-75 or one T-185 flask
14. For other MSC cells, optimal plating density will have to empirically determined and will be cell line specific.
15. Place Tissue Culture Flask/Dish in 5% CO_2 Tissue Culture Incubator at 37°C
16. Change medium on flasks on the next day.

B. Growing Mesenchymal Stem Cells:

1. *Medium Change (Culture Medium should be changed every 3-4 days)*
   a. Warm Complete Growth Medium to 37 °C by placing in 37°C H_2O bath or in Tissue Culture Incubator
   b. Spray down bottle containing growth medium with 70% EOH before placing in Tissue Culture Hood
   c. Remove medium from T-75 flask containing Mesenchymal Stem Cells
   d. Add 12-15 ml of fresh Complete Growth Medium
   e. Discard used medium appropriately

2. *Passaging Mesenchymal Stem Cells*
   When MSCs become 70- 80% confluent they are ready to be split. If allowed to over-grow, these cells will lay down a matrix and start to differentiate. As a result, the cells will peel off of the plastic which markedly reduces the ability to passage them.

   a. Warm Mesenchymal Complete Growth Medium and trypsin solution to 37 °C by placing in 37 °C H_2O bath or in Tissue Culture Incubator
   b. Spray down bottle containing growth medium, and trypsin bottle with 70% EtOH before placing in Tissue Culture Hood
   c. Remove medium from T-75 flask containing Mesenchymal Stem Cells
   d. Gently wash flask with 5-10 ml of sterile 1X PBS (Ca^{2+} and Mg^{2+} free)
   e. Remove PBS
   f. Add 3 ml of Trypsin solution to each flask place at 37°C in Tissue Culture Incubator for 3-5 minutes (until cells are no longer attached to plate, this should take no longer than 5 minutes).
   g. Add 5 ml of warm Complete Growth Medium to flask
   h. Transfer cells to 15 ml conical tube
   i. Centrifuge 15 ml conical tube at 200 x g for 3 minutes at room temperature

j. Remove supernatant gently to avoid disturbing cell pellet
k. Resuspend cell pellet in 2 ml of fresh Complete Growth Medium
l. Count cells on hemocytometer (per standard protocol)
m. For Trevigen's Rat MSC: Passage cells at a density of $5.4x10^3$ cell/cm^2. For a T-75 flask add $4.05x10^5$ cells in 12-15 ml of complete growth medium
n. For other MSC cells, optimal plating density will have to empirically determined and will be cell line specific

Note: One flask of 70-80% confluent cells should be enough to be plated into 1-3 T-75 flasks.

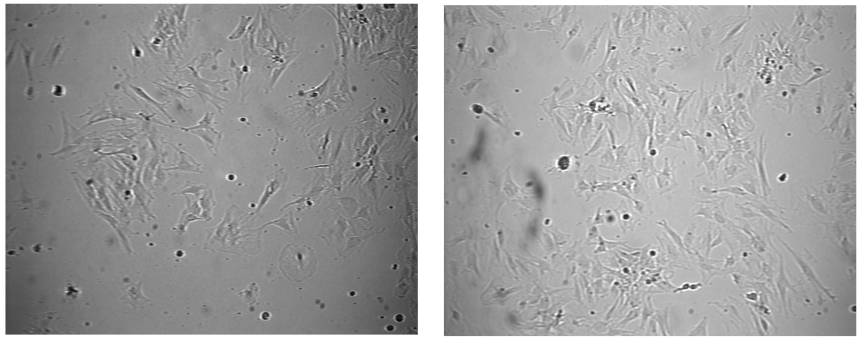


Figure 1: 10X Bright Field Images of Rat Mesenchymal Stem Cells.

C. Freezing Cells

1. Warm Complete Growth Medium and trypsin solution to 37 °C by placing in 37 °C H_2O bath or in Tissue Culture Incubator
2. Make Freeze Medium (see Section V.2), adjust volume according to volume needed (*Will be mixed 1:1 with Complete Growth Medium)*
3. Spray down bottles containing Complete Growth Medium, trypsin, and the Freeze Medium tube with 70% EtOH before placing in Tissue Culture Hood
4. Remove medium from T-75 flask containing Mesenchymal Stem Cells
5. Gently wash flask with 5-10 ml of sterile 1X PBS (Ca^{2+} and Mg^{2+} free)
6. Remove PBS
7. Add 3 ml of Trypsin solution to each flask place at 37 °C in Tissue Culture Incubator for 2-3 minutes (until cells are no longer attached to the plate, which should take no more than 5 minutes).
8. Add 5 ml of Complete Growth Medium to flask
9. Transfer cells to 15 ml conical tube
10. Centrifuge 15 ml conical tube at 200 x g for 3 minutes at room temperature
11. Remove supernatant gently to avoid disturbing cell pellet
12. Resuspend cell pellet in 2 ml of Complete Growth Medium
13. Count cells on hemocytometer (per standard protocol)
14. Dilute cells to a desired concentration for freezing.

Notes: We recommend a concentration of no less than $1x10^6$ cell/ml. This concentration is enough to seed one T-75 flask (remember, the cells will be diluted 1:1 with Freeze Medium). One T-75 flask will provide enough cells for 1-3 vials of $5x10^5$ cells/vial.

15. Add an equal volume of Freeze Medium to the cells, mix gently
16. Aliquot 1 ml of cells into labeled cryovials
17. Place on ice of 15-30 minutes
18. Transfer cells to -80°C freezer and incubate overnight
19. Transfer to liquid nitrogen freezer for long term storage

Note: Vapor phase is recommended to ensure viability

D. Osteogenic Differentiation of Mesenchymal Stem Cells

When MSCs become 70- 80% confluent they are ready to be split. If allowed to over-grow, these cells will lay down a matrix and start to differentiate, thereby allowing them to peel off which markedly reduces the ability to passage them.

Day before plating of the cells, coat 24-well plate with Cultrex® Collagen I (Rat)
a: Add 500 µl of Collagen Solution for Coating (sect V.4) to each well
b: Place plate at 37°C CO_2 incubator overnight
Note: If not working in a 24 well plate, adjust coating volume accordingly.

1. *Plating Cells*
   a. Warm Complete Growth Medium and trypsin solution to 37°C by placing in 37°C H_2O bath or in Tissue Culture Incubator
   b. Spray down bottle containing Complete Growth Medium, and trypsin bottle with70% EtOH before placing in hood
   c. Remove medium from T-75 flask containing Mesenchymal Stem Cells
   d. Gently wash flask with 5-10 ml of sterile 1X PBS (Ca^{2+} and Mg^{2+} free)
   e. Remove PBS
   f. Add 3 ml of Trypsin to each flask and place at 37°C in Tissue Culture Incubator for 3-5 minutes (until cells are no longer attached to plate, which should take no more than 5 minutes).
   g. Add 5 ml of warm Complete Growth Medium to flask
   h. Transfer cells to 15 ml conical tube
   i. Centrifuge 15 ml conical tube at 200 x g for 3 minutes at room temperature
   j. Remove supernatant gently to avoid disturbing cell pellet
   k. Resuspend cell pellet in 2 ml of fresh Compete Growth Medium
   l. Count cells on hemocytometer (per standard protocol)
   m. Remove medium from Collagen coated 24-well plate
   n. For Trevigen's Rat MSC: Plate cells at 5,000 cells/cm^2. For a 24-well plate add 10,000 cells/well in 0.5 ml of complete growth medium per well.
   o. For HMSC [e.g. Zen-Bio, Inc cat# ACS-F], Plate cells at 30,000 cells/cm^2. For a 24-well plate add 60,000 cells/well in 0.5 ml of complete growth medium per well.
   p. For other MSC, initial plating concentration will have to be optimized for endogenous matrix deposition by bone marrow derived MSC.
   Note: If not using 24-well plate, adjust medium volumes accordingly. Components are sufficient to differentiate one 24-well plate using the stated medium volumes.
   q. Incubate overnight at 37°C

2. *Differentiation*
   a. Observe the plates under phase microscope to ensure that the mesenchymal stem cells have attached to the plate.
   b. Medium needs to be changed every 3-4 days for 4 days.
   c. Procedure:
      i. Day 1
         1. Label wells for Differentiated or Undifferentiated control groups
         2. For Differentiation: Remove Complete Growth Medium and add 0.5 ml of freshly prepared Osteogenic Differentiation Medium (sect. V.3) to each well
         3. For Undifferentiated controls: Remove Complete Growth Medium and add 0.5 ml of fresh Complete Growth Medium (V.1) to control wells
      ii. Days 4, 7, 10, 13 (if extending culture to 21 days continue changing medium every 3-4 days)
         1. For Differentiation: Remove spent Differentiation Medium and add 0.5 ml of freshly prepared Osteogenic Differentiation Medium (section V.3) to each well
         2. For Undifferentiated controls: Remove growth medium and add 0.5 ml of Complete Growth Medium (V.1) to control wells

      Note: Confluent cultures undergoing osteogenic differentiation tend to lift off or "peel." Osteogenic differentiation occurs within 7 days of growth in osteogenic medium. Peeling can begin to occur within 12 days in culture. Osteogenic differentiation induction can be extended up to 21 days depending on cell number and reduced growth rate.

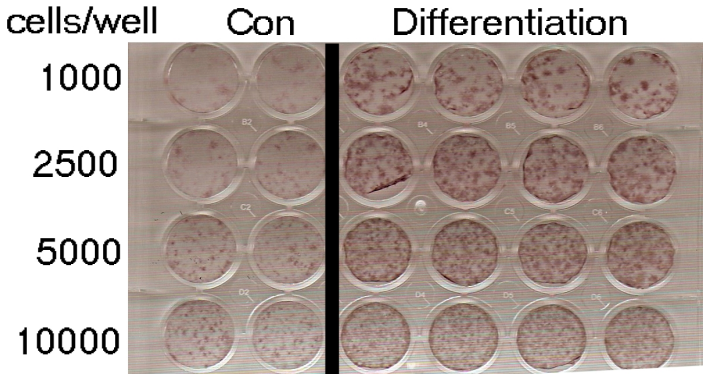


Figure 2: Rat MSCs [Trevigen cat# 5000-001-01] grown on Rat Tail Collagen I-Coated wells for 14 days in Osteogenic Differentiation Medium, fixed and stained with Alizarin Red S

   d. Evaluation of Osteogenic Differentiation: Alizarin Red S staining (Day 4):
      i. Remove the media from each well
      ii. Gently wash wells with 1 ml of 1X PBS
      iii. Remove PBS
      iv. Add 0.5 ml of 10% Formalin in PBS to each well
      v. Incubate plate at room temperature for 30 minutes
      vi. Remove 10% Formalin
      vii. Wash each well with 1 ml of ddH_20
      viii. Add 0.5 ml of ddH_20 to each well and incubate plate for 5 minutes
      ix. Remove ddH_20
      x. Add 0.5 ml of Alizarin Red S to each well

      xi. Incubate plate for 20 minutes at room temperature with gentle shaking (shaking optional)
      xii. Remove Alizarin Red S solution
      xiii. Wash wells twice with 1 ml of ddH_20
      xiv. Remove ddH_20 and let the wells air dry.

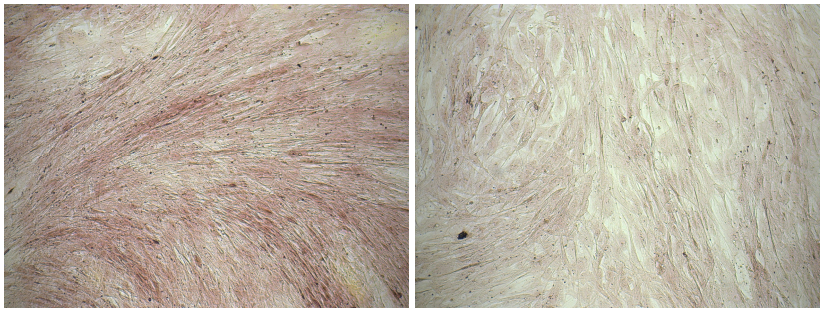


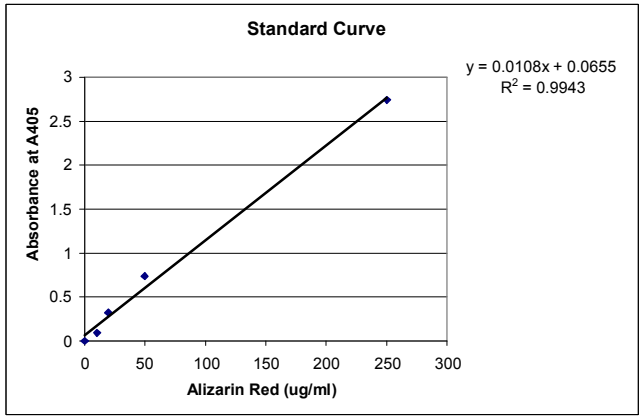
Figure 3: HMSC [Zen-Bio, Inc, cat# ACS-F] grown on Rat Tail Collagen I-Coated wells for 14 days in Osteogenic Differentiation Medium (left), grown in control medium (right), fixed and stained with Alizarin Red S

   e. Quantification of Alizarin Red S Stain
      i. Standard Curve
         1. Prepare Samples according to the following table. Use 1.7 ml tubes

	Standard (concentration of Alizarin Red S stain)	Alizarin Red Stain	Solubilization Solution
A	1 mg/ml	50 µl Stock Solution	950 µl
B	500 µg/ml	500 µl of A	500 µl
C	250 µg/ml	500 µl of B	500 µl
D	100 µg/ml	400 µl of C	600 µl
E	50 µg/ml	500 µl of D	500 µl
F	25 µg/ml	500 µl of E	500 µl
G	10 µg/ml	400 µl of F	600 µl
H	5 µg/ml	500 µl of G	500 µl
I	0 µg/ml	0 µl	500 µl

         2. Transfer 150 µl of standard solutions B-I to a clear 96-well plate in triplicate.
      ii. Extraction of Alizarin Red Stain from wells
         1. Add 500 µl of Stain Solubilization Solution to each well of the 24 well plate
         2. Incubate plate at room temperature for 10 min.
         3. Transfer 150 µl of sample to the 96-well clear plate in triplicate
      iii. Read absorbance of standards and extracted Alizarin Red Stain on a plate reader at 405 nm
      iv. Calculate the Alizarin Red S present in each sample relative to the standard curve:

1. Plot the standards with Absorbance at A_{405} on the Y-axis and concentration of Alizarin Red S standard on the X-axis, if using Excel, use the X-Y graph feature. Be sure to subtract the 0 µg/ml (I) value from all standard (and sample) values.
2. Insert a trendline and determine the equation of the standard curve line. Your trendline should have an R^2 value $\geq$ 0.975
3. Take sample readings at A_{405} (use 150 µl per triplicate sample). Using the equation from the standard curve, calculate the amount of Alizarin Red S present in the samples.
4. Plot the amount of Alizarin Red S present in the samples and controls on a bar graph.
5. The amount of Alizarin Red S stain present in a sample is relative to the amount of differentiation occurring in the culture. By day 14 of the assay, cells will be confluent so the amount of stain is representative of the differentiation state; not the number of cells present.



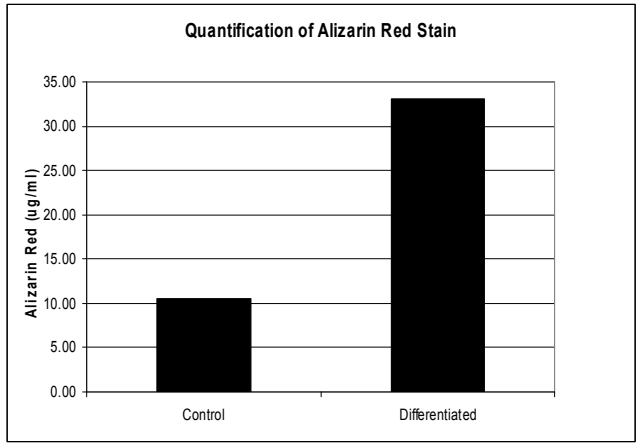
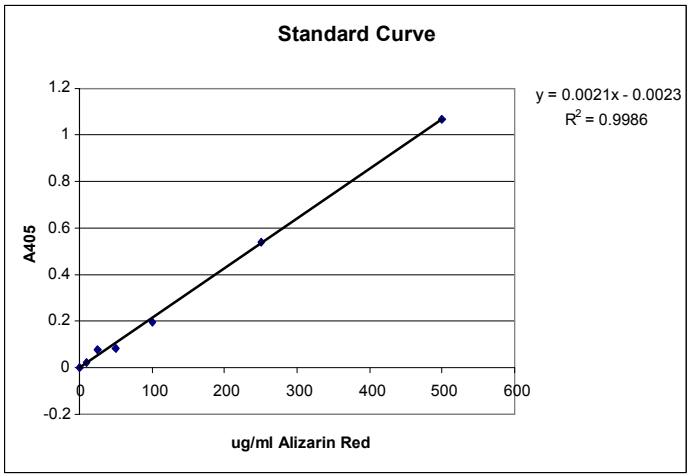


Figure 5: Quantitation of Alizarin Red Staining of Rat Mesenchymal Stem Cells [Trevigen, Inc cat# 5000-001-01] cultured in Osteogenic Differentiation Medium for 14 days



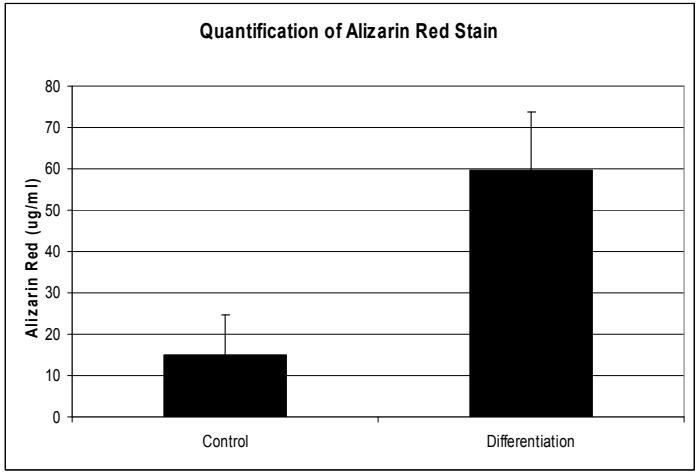


Figure 6: Quantitation of Alizarin Red Staining of Human Mesenchymal Stem Cells [Zen-Bio, Inc cat# ACS-F] cultured in Osteogenic Differentiation Medium for 14 days

VII. References

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VIII. Troubleshooting

PROBLEM	CAUSE	ACTION
Poor Viability from initial freeze	To rough in thawing of cells	Ensure medium is added slowly to re-equilibrate the MSC from freeze medium Ensure cells were removed from freeze medium immediately after vial has been thawed Ensure Vial of cells was thawed at 37 °C Fresh media was pre-warmed to 37 °C
Poor proliferation	Medium and Fetal Bovine Serum not optimized for support of MSC growth	For RMSC: Use Qualified RMSC Medium and FBS from Trevigen For other MSC: Try different lots of medium and fetal bovine serum or purchase qualified medium and serum appropriate for cell origin.
	Tissue Culture Labware not ideal for MSC	Use Corning or Nunc Treated Labware
	Frequency of Medium Change	Ensure medium is changed every 3-4 days Ensure pH of fresh medium has not changed.
	CO_2 Incubator not humidified No gas exchange is allowed by flask	Add sterile water to CO_2 incubator per manufacturer's instructions Ensure cap is loosened to allow air gas or use vented flask
	Cells were allowed to become over confluent and lay down matrix	Extend time in trypsin Tirtrate cells to remove as many cells as possible from matrix Remove visual matrix aggregated from tube before spinning (will reduce cell recovery) Pass cell suspension through cell strainer (will reduce cell recovery)

PROBLEM	CAUSE	ACTION
Cells were clumpy after passaging, limited recovery of single cells.	Cells were allowed to become over confluent and lay down matrix	Extend time in trypsin Titrate cells to remove as many cells as possible from matrix Remove visual matrix aggregated from tube before spinning (will reduce cell recovery) Pass cell suspension through cell strainer (will reduce cell recovery)
Contamination of Cells	Contaminated Medium	To prevent contamination, filter medium through a 0.22 µm filter before use *Never use contaminated medium once cloudy or after microorganisms are visible under the microscope.*
	Improper Aseptic Technique	Spray down hands, reagents and hood with 70% ethanol before opening any flasks
	Hood is working improperly	Check to make sure blower is on and functioning Ensure hood is currently certified Wipe down hood with 70% ethanol
	Contaminated CO_2 Incubator.	Ensure CO_2 incubator is free of microbial growth
Poor Cell Recovery from flask (for cell growth)	Too high seeding density	Passage cells at lower confluency
No Osteogenic Differentiation or low levels of differentiation	Serum	Cells were grown in serum that did not support undifferentiated growth, for RMSC use Trevigen's Qualified RMSC FBS. For other MSC try different fetal bovine serum
	Medium was not prepared properly	Ensure the supplements were added to medium in proper amounts
	Change basal medium type	Ensure basal medium contains calcium
	Too long between medium changes	Ensure medium is being changed every 3-4 days
	Cell were plated too sparse	Plate cells at a higher density
Osteogenic differentiation present in control medium	Cells plated too high density	Plate cells at lower density
Cells peeling from wells	Cells over-confluent	Plate cells at a lower concentration
High background in control wells for during quantification	Presence of non-specific stain in the well	Increase number of washes

IX. Related Products Available From Trevigen

Contact Trevigen for details of our unique product line for studying DNA damage and repair. All of Trevigen's kits include highly qualified enzymes, substrates.

Differentiation:

Catalog #	Description	Size
5000-001-K	Cultrex® Rat Mesenchymal Stem Cell Starter Kit	1 kit
5000-001-R	Cultrex® Rat Mesenchymal Stem Cell Replenisher Kit	1 kit
5000-001-01	Cultrex® Rat Mesenchymal Stem Cells	1 vial
5010-024-K	Cultrex® Adipogenic Differentiation Kit	24 samples

3D Culture Kits:

Catalog #	Description	Size
3445-096-K	Cultrex® 3D Culture BME Cell Proliferation Assay Kit	96 tests
3446-096-K	Cultrex® 3D Culture Laminin I Cell Proliferation Assay	96 tests
3447-096-K	Cultrex® 3D Culture 96 Well Collagen I Cell Prolif Assay	96 tests
3448-020-K	Cultrex® 3D Culture Cell Harvesting Kit	96 tests

Invasion/Migration Kits:

Catalog#	Description	Size
3455-024-K	Cultrex® 24 Well BME Cell Invasion Assay	24 inserts
3460-024-K	CultreCoat® 24 Well BME-Coated Cell Invasion Assay	24 inserts
3465-096-K	Cultrex® 96 Well Cell Migration Assay	96 samples
3465-024-K	Cultrex® 24 Well Cell Migration Assay	12 samples
3455-096-K	Cultrex® 96 well BME Cell Invasion Assay	96 samples
3456-096-K	Cultrex® 96 well Laminin I Cell Invasion Assay	96 samples
3457-096-K	Cultrex® Collagen I Cell Invasion Assay	96 samples
3458-096-K	Cultrex® Collagen IV Cell Invasion Assay	96 samples

Accessories:

Catalog#	Description	Size
3415-001-02	Cultrex® Human BME, PathClear®	1 ml
3432-005-02	Cultrex® BME, PathClear®	5 ml
3432-005-01	Cultrex® BME without Phenol Red	5 ml
3431-005-01	Cultrex® BME with Phenol Red; Reduced Growth Factor	5 ml
3433-005-01	Cultrex® BME; no Phenol Red; Reduced Growth Factor	5 ml
3430-005-02	Cultrex® BME with Phenol Red, PathClear®	5 ml
3431-005-02	Cultrex® BME with Phenol Red, Reduced Growth Factor PathClear®	5 ml
3400-010-01	Cultrex® Mouse Laminin I	1 mg
3440-100-01	Cultrex® Rat Collagen I	100 mg
3410-010-01	Cultrex® Mouse Collagen IV	1 mg
3420-001-01	Cultrex® Human Fibronectin, PathClear®	1 mg
3416-001-01	Cultrex® Bovine Fibronectin, NZHD*	1 mg
3421-001-01	Cultrex® Human Vitronectin, PathClear®	50 µg
3417-001-01	Cultrex® Bovine Vitronectin, NZHD	50 µg
3438-100-01	Cultrex® Poly-L-Lysine	100 ml
3439-100-01	Cultrex® Poly-D-Lysine	100 ml
3445-048-01	Cultrex® 3-D Culture Matrix™ BME	15 ml
3446-005-01	Cultrex® 3-D Culture Matrix™ Laminin I	5 ml
3447-020-01	Cultrex® 3-D Culture Matrix™ Collagen I	100 mg
3430-005-01	Cultrex® BME with Phenol Red	5 ml
3437-100-K	Cultrex® Cell Staining Kit	100 ml
3450-048-05	CellSperse™	15 ml

*New Zealand Herd Derived

Please

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