CULTREX® **Instructions**

For Research Use Only. Not For Use In Diagnostic Procedures

Mesenchymal Stem Cell

Adipogenic Differentiation Kit

Cat # 5010-024-K

Reagents for differentiating Mesenchymal Stem Cells into Adipocytes

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Introduction

Adipogenesis is the process by which fat is formed.[1] Fat formation plays a role in obesity, cardiovascular disease, and metabolic disorders.[1] It also has a role in the maintenance of the bone marrow cavity. Adipocytes are derived from mesenchymal stem cells (MSC), which are a self-renewing population of multipotent cells present in bone marrow and many other adult tissues that can differentiate into multiple lineage-specific cells that form bone, fat, cartilage, muscle and tendon.[2-5] Differentiation of primary cells, specifically MSC, into adipocytes can be a useful tool for understanding the mechanisms involved in adipogenesis leading eventually to interventions designed to prevent obesity.

Trevigen's adipogenic differentiation kit follows traditional methods of adipogenic differentiation,[6] by growing MSC in medium supplemented with insulin, isobutyl methyl xanthine (IBMX), indomethacin and dexamethasone. The kit will induce adipogenic differentiation of Trevigen's Rat MSC (cat# 5000-001-01) or human MSC when grown in the appropriate Qualified medium supplemented with Qualified FBS within 14 days. Adipogenic differentiation is detected by staining with Oil Red O. Oil Red O will stain lipid containing vacuoles in mesenchymal stem cells that have undergone adipogenic differentiation.

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
IBMX	65 µl	-20°C	5010-024-02
Insulin	110 µl	4°C*	5010-024-03
Indomethacin	330 µl	-20°C	5010-024-04
Oil Red O[2]	15 ml	RT in dark*	5010-024-05

Notes:
1: Reagents are sufficient to differentiate one 24 well plate
2: Enough stain 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. Spectrophotometer (reads wavelength of 500 nm)

Reagents

1. Mesenchymal Stem Cells (Trevigen's RMSC cat# 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 Fetal Bovine Serum (for RMSC cat# 5000-050-02) or equivalent
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. 0.45 µm Syringe Filter
6. 60 ml Syringe
7. 1 - 200 µl and 200 - 1000 µl pipette tips
8. 1, 5 and 10 ml serological pipettes
9. gloves
10. plastic cuvette
11. 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 is 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 Complete Growth Medium before use

3. Adipogenic Differentiation Medium*

For 13 ml of Medium

Mesenchymal Complete Growth Medium (From V.I.):	12.96 ml
Dexamethasone	1.3 µl
IBMX	13 µl
Indomethacin	65 µl
Insulin	13 µl

Invert several times to Mix.
*Make up Fresh Prior to use
Ensure media is at room temperature or 37°C prior to use

4. Adipogenic Maintenance Medium*

For 13 ml of Medium

Mesenchymal Complete Growth Medium (From V.I.):	12.99 ml
Insulin	13 µl

Invert several times to Mix.
*Make up Fresh Prior to use
Ensure media is at room temperature or 37°C prior to use

5. Oil Red O Working Solution

For 25 ml of stain (sufficient to stain two 24 well plates)

Oil Red O	15 ml
1X PBS	10 ml

Mix by inverting
Filter though 0.45 µm filter (syringe or filter unit)
Let stand for 20 min @ RT in dark before use.

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 (e.g. Rat MSC cat# 5000-001-01) from liquid nitrogen freezer
4. Thaw frozen MSC 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 with 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. Wash ampoule with 1 ml of warm Complete Growth Medium using a 5 ml pipette
8. Transfer the contents from step 8 to the 15 ml conical tube containing thawed cells dropwise, gently swirling to mix between drops.
9. Add drop-wise to 15 ml conical tube containing cells, gently swirling to mix between drops
10. Add 1 ml of warm medium to 15 ml conical tube containing cells, gently swirling to mix between drops
    a. Total Volume should be about 3 ml
11. Centrifuge 15 ml conical tube at 250 x g for 5 minutes at room temperature
12. Remove supernatant gently to avoid disturbing cell pellet
13. Resuspend cell pellet in 1 ml of fresh Complete Growth Medium
14. Count cells on hemocytometer (per standard protocol)
15. 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
    a. Recommend Corning® Tissue Culture Treated Plastic
    b. One vial is sufficient to seed two T-75 or one T-185 flask
16. For other MSC cells, optimal plating density will have to empirically determined and will be cell line specific
17. Place Tissue Culture Flask/Dish in 5% CO_2 Tissue Culture Incubator at 37°C
18. Change medium in 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% EtOH 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 cells became 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 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 to each flask and place at 37°C in Tissue Culture Incubator for 3-5 minutes (until cells are no longer attached to plate, it should no longer than 5 minutes) .
   i. Add 5 ml of warm Complete Growth Medium to flask
   ii. Transfer cells to 15 ml conical tube
   iii. Centrifuge 15 ml conical tube at 200 x g for 3 minutes at room temperature
   iv. Remove supernatant gently to avoid disturbing cell pellet
   v. Resuspend cell pellet in 2 ml of fresh medium
   vi. Count cells on hemocytometer (per standard protocol)
   vii. For Trevigen's Rat MSC: Passage cells at a density of $5.4x10^3$ cell/cm^2. For a T-75 flask add 4.05×10^5 cells in 12-15 ml of Complete Growth Medium
   viii. 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 able to be split into 2-3 T-75 flasks.

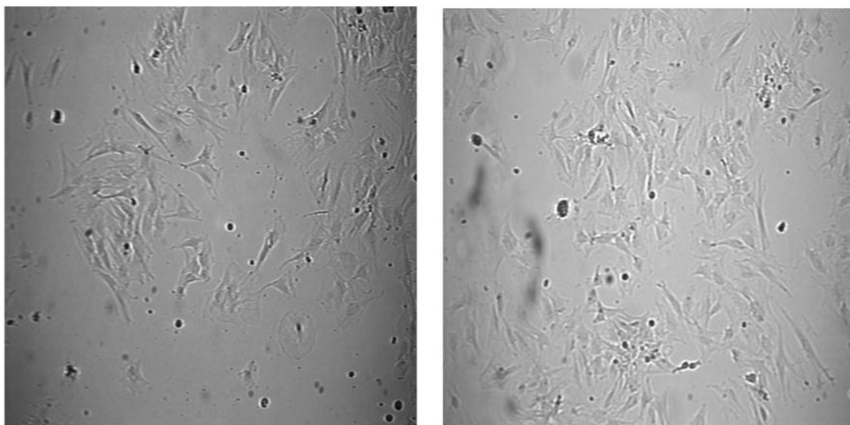

Figure 1: 10X Bright Field Images of Rat Mesenchymal Stem Cells.

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 need (*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 from incubator
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 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 longer 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 1 x 10^6 cells/ml. This concentration is enough to seed one T-75 flask (remember, the cells will be diluted 1:1 with freeze medium).

15. Add equal volume of Freeze Medium to the cells, mix gently
16. Aliquot 1 ml of cells into labeled cryovials
17. Place on ice for 15-30 minutes
18. Transfer 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. Adipogenic Differentiation of Mesenchymal Stem Cells

When cells became 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.

1. <u>Passaging Cells</u>
   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 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 to each flask and place at 37°C in Tissue Culture Incubator .for 3-5 minutes (until cells are no longer attached to plate, it should take no more than 5 minutes)
   g. Add 5 ml of Complete Growth Medium to flask
   h. Transfer cells to 15 ml conical tube
   i. Centrifuge 15 ml tube conical 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: 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.
   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.
n. For other MSC: Optimal plating density will need to be determined. (e.g. For Human adipose derived MSC [Zen-Bio, Inc cat# ACS-F], a cell density of 40,000 cell/cm^2 is recommended.) For a 24-well plate add ~80,000 cells/well in 0.5 ml of Complete Growt Medium
o. Let cells grow overnight at 37°C

2. *Differentiation*

a. Observe the plates under phase microscope to ensure that the mesenchymal stem cells have attached to plate, if Trevigen's RMSC are at least 30% confluent or human adipose derived MSC are 90% confluent, then proceed to Induction, if not, wait 24 hours, then proceed. For other MSC, seeding density may need to be optimized.

b. Medium needs to be changed every 3-4 days for 14 days.

c. Observe cells daily. Within one week of inducing adipogenic differentiation, the formation of lipid droplets should be evident within the cultures (Figure 2).

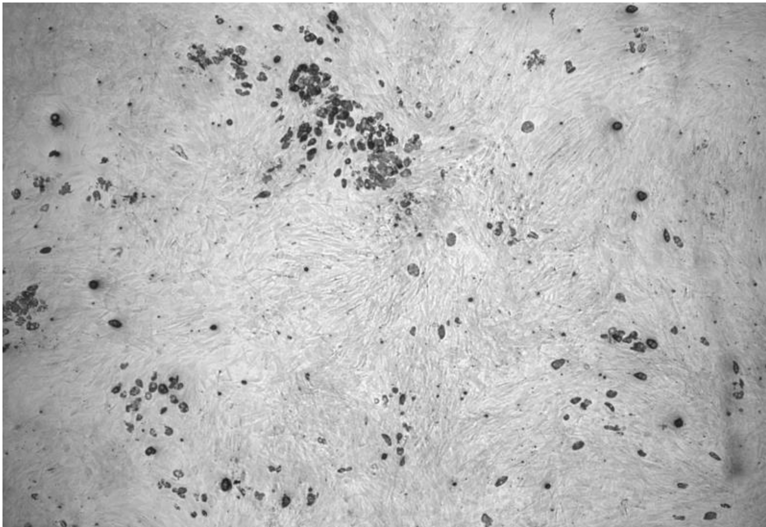

Figure 2: Bright Field image of Lipid Droplets formed by differentiated Rat Mesenchymal Stem Cells. Magnification is 10X.

d. Induction:
   i. Day 1 - Induction #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 Adipogenic Differentiation Medium (section V.3) to each well
3. For Undifferentiated controls: Remove growth medium and add 0.5 ml of fresh Complete Growth Medium (V.1) to control wells

ii. Day 4 - Induction #2
1. For Differentiation: Remove differentiation medium and add 0.5 ml of freshly prepared Adipogenic Differentiation Medium (section V.3) to all differentiation wells
2. For Undifferentiated controls: Remove growth medium and add 0.5 ml of Complete Growth Medium (section V.1) to control wells

iii. Day 7 - Induction #3
1. For Differentiation: Remove differentiation medium and add 0.5 ml of freshly prepared Adipogenic Differentiation Medium (section V.3) to all differentiation wells
2. For Undifferentiated controls: Remove growth medium and add 0.5 ml of Complete Growth Medium (V.1) to control wells

e. Maintenance:

i. Day 10 (or 11) - Maintenance #1
1. For differentiated wells: Remove differentiation medium and add 0.5 ml of freshly prepared Adipogenic Maintenance Medium (section V.4) to each maintenance well
2. For Undifferentiated controls: Remove growth medium and add 0.5 ml of Complete Growth Medium (V.1) to control wells

Note: If using Trevigen RMSC, Qualified RMSC medium and serum or human adipose derived MSC, lipid droplets should be clearly present. If using other MSC or growing conditions, an additional round of induction may be required to induce adipogenic differentiation (i.e. formation of lipid droplets). Repeat Induction procedure in section d.2. For prolonged culture of differentiated and control cell populations, repeat the maintenance procedure (e. above) every three-four days.

f. Confirmation of Adipogenic Differentiation: Oil Red O staining (Day 14-Day17):

i. Remove medium from each well
ii. Gently wash with 1 ml of 1X PBS
iii. Remove PBS
iv. Add 0.5 ml of 10% Formalin in PBS to each well
v. Incubate the plate at room temperature for 30 minutes
vi. Prepare Oil Red O working solution (see section V.5)
vii. Remove 10% Formalin
viii. Wash twice with 1 ml per well of 1X PBS
ix. Add 0.5 ml of Oil Red O working solution to each well
x. Incubate the plate for 30 minutes with gentle shaking (shaking optional)
xi. Remove Oil Red O solution
xii. Wash each well twice with 1X PBS
xiii. Add 500 µl of 1X PBS in each well and image the wells. This will ensure integrity of the lipid droplets.
xiv. Remove PBS and let the plate air dry.

Note: *Lipid droplets will flatten out with drying and thereafter resolution of individual droplets will be lost.*

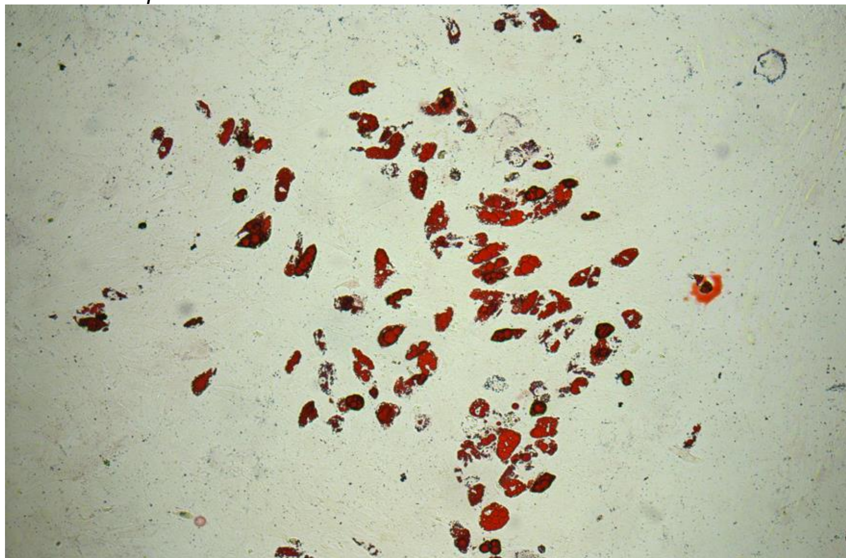

Figure 3: Rat MSC grown for 14 days in Adipogenic Differentiation Medium, fixed and stained with Oil Red O. Lipid droplet formation was present across the entire plate. Images were captured at 10X magnification.

Figure 4: Control Rat MSC grown for 14 days in Complete Growth Medium, fixed and stained with Oil Red O. No lipid droplets were present in the culture. Images were captured at 10X magnification.

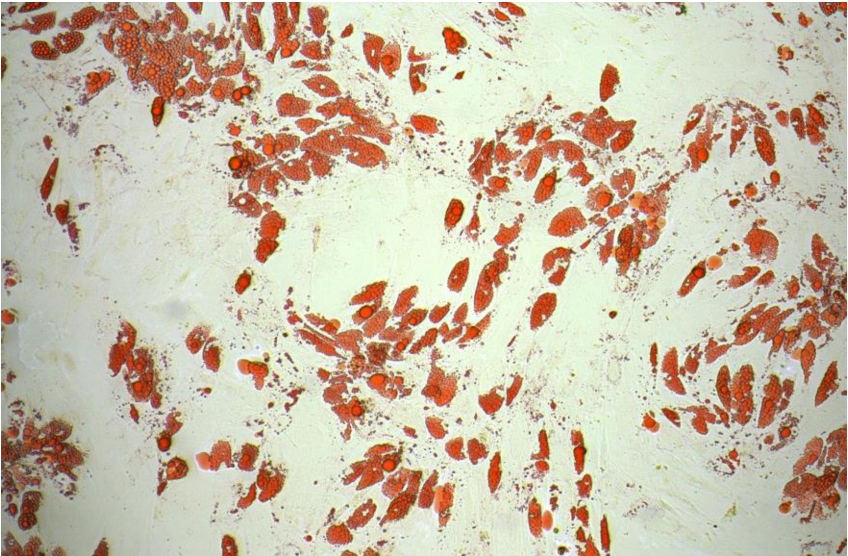

Figure 5: Human Adiopse Derived MSC [Zen-Bio, Inc cat# ACS-F] grown for 14 days in Adipogenic Differentiation Medium, fixed and stained with Oil Red O. Lipid droplet formation was present across the entire plate. Images were captured at 10X magnification.

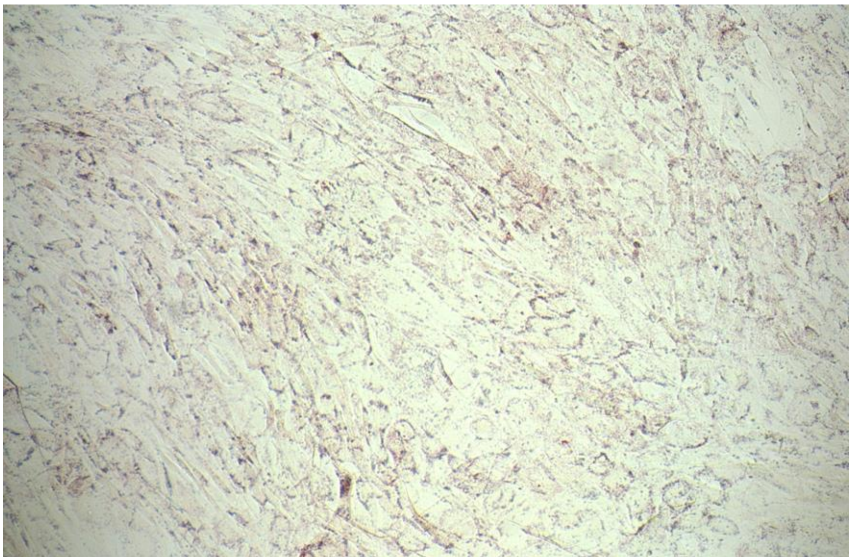

Figure 6: Control Human Adipose Derived MSC [Zen-Bio, Inc cat# ACS-F] grown for 14 days in Complete Growth Medium, fixed and stained with Oil Red O. No expanded lipid droplet formation was observed in the culture. Images were captured at 10X magnification.

E. **Quantification of Oil Red O Stain**

1. Standard Curve
   a. Prepare Standards according to the following table in 1.7 ml tubes:

	Standard (concentration of Oil Red O stain)	Oil Red O Stain	Isopropanol
A	500 µg/ml	250 µl of working solution (V.5)	1250 µl
B	250 µg/ml	750 µl of A	750 µl
C	100 µg/ml	600 µl of B	900 µl
D	50 µg/ml	750 µl of C	750 µl
E	0 µg/ml	0 µl	750 µl

   b. Transfer 750 µl of standard solutions A-E to disposable plastic cuvettes.

   c. Extract stain
      i. Add 750 µl of Isopropanol to each well of the 24-well pate
      ii. Incubate at room temperature for 10 minutes with shaking
      iii. Transfer 750 µl of sample to a plastic cuvette

   d. Read the absorbance of the standard curve and samples at an absorbance of 500 nm using the 0 µg/ml (E) standard to blank the spectrophotometer.

   e. Calculate the Oil Red O present in each sample relative to the standard curve. If the absorbance of any of your samples is greater than the absorbance of the highest point on your standard curve, dilute the sample 1:2 with isopropanol and read again.

      i. Plot the standards with Absorbance at A_{500} on the Y-axis and concentration of Oil Red O standard on the X-axis. If using Excel use the X-Y graph feature.
      ii. Insert a trendline and determine the equation of the standard curve line. Your trendline should have an R^2 value $\geq$ 0.975
      iii. Using the equation from the standard curve, calculate the amount of Oil Red O present in the samples. Multiple by the dilution factor (if any dilution was used).
      iv. Plot the amount of Oil Red O present in the samples and controls on a bar graph.
      v. The amount of Oil Red O stain present in a sample is relative to level of lipid droplet formation in your culture.

VII. References

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5. Li Yi, McIntosh K, Chen J, Zhang C, Gao, Q, Borneman J, Ragniski K, Mitchell J, Shen L, Zhang J, Lu D, Chopp M. Allogeneic bone marrow stromal cells promote glial-axonal remodeling without immunologic sensitization after stroke in rats. *Experimental Neurology* 198(2):313-25.
6. Reger RL, Tucker AH, Wolfe MR. 2008. Differentiation and Characterization of Human MSCs. In Prockop DJ, Phinney DG, Bunnell BA (eds) *Methods in Molecular Biology Mesenchymal Stem Cells:Methods and Protocols* vol 449. Humana Press, Totawa, NJ, USA pp 93-107.

VIII. Troubleshooting

PROBLEM	CAUSE	ACTION
Poor Viability from initial freeze	Too 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 MSC 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	Add sterile water to CO_2 incubator per manufacturer's instructions
	No gas exchange is allowed by flask	Ensure cap is loosened to allow air gas or use vented flask

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 Adipogenic 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
	Cells take longer to differentiate	Repeat growth in induction medium
	Precipitation of Indomethacin stock solution	Ensure no precipitation is visible in the Indomethacin solution, if present, heat solution @ 37°C to solubilize
	Too long between medium changes	Ensure medium is being changed every 3-4 days
No expansion of lipid droplets after induction	Insulin not present in medium	Ensure Insulin was added to Adipogenic Maintenance Medium
	Insulin stored improperly	Ensure that storage of insulin was done at 4°C and not undergone repeated freeze thaw. To store long-term, aliquot into smaller volumes then store at -80 °C.

PROBLEM	CAUSE	ACTION
Cells peeling from wells	Cells over-confluent	Plate cells at a lower concentration Coat wells with 10 µg of type I rat collagen (cat #3440-100-01), repeat assay
Indomethacin stock has precipitates in it	Properties Inherent with Indomethacin solution	Warm Solution to 37°C before addition to media
IBMX precipitating when added to Adipogenic medium	Properties Inherent with IBMX	Heat medium to 37°C If still visible, no additional action is necessary. Will not adversely effect differentiation of the mesenchymal stem cells
Precipitation in Oil Red O working solution after filtering	Insufficient Filtering	Filter through 0.22 µm Filter When adding to syringe for filtering, carefully pour working solution, do not add any precipitated dye particles present after mixing with PBS
Control wells have high background staining	Insufficient washing	Wash longer with PBS after staining, critical all non-specific staining removed before quantification

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, buffers, full instructions for use, and a synopsis specific for your kit.

Stem cell Products:

Catalog#	Description	Size
5000-001-K	Cultrex® Rat Mesenchymal Stem Cell Starter kit	1 vial
5000-001-R	Cultrex® Rat Mesenchymal Stem Cell Replenisher Kit	1 kit
5000-001-01	Cultrex® Rat Mesenchymal Stem Cells	1 vial
5011-024-K	Cultrex® MSC Osteogenic 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

Catalog#	Description	Size
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
3471-096-K	*In vitro* Angiogenesis Assay Endothelial Cell Invasion	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
3400-010-02	Cultrex® Mouse Laminin I, PathClear®	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

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes or for use in humans.

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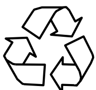

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