CULTREX® Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

Rat Mesenchymal Stem Cell Starter Kit

Cat # 5000-001-K

Primary Mesenchymal Stem Cells isolated and purified from Rat Bone Marrow plus qualified medium and serum to support undifferentiated growth

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I. Introduction

Mesenchymal Stem Cells (MSC), also known as marrow stromal cells, are a self-renewing population of multipotent cells present in bone marrow and many other adult tissues.[1,2] MSC can be isolated from bone marrow by adherence to plastic,[1-4] and can differentiate into multiple lineage-specific cells that form bone, fat, cartilage, muscle, neuronal cells and tendon.[1-4] Due to their multilineage potential, they can be useful tools for a wide range of therapeutic and basic research, including transplantation studies and studies examining the repair of cardiac tissue, bone, cartilage, and tendons often using 3-D matrices.[1,4]

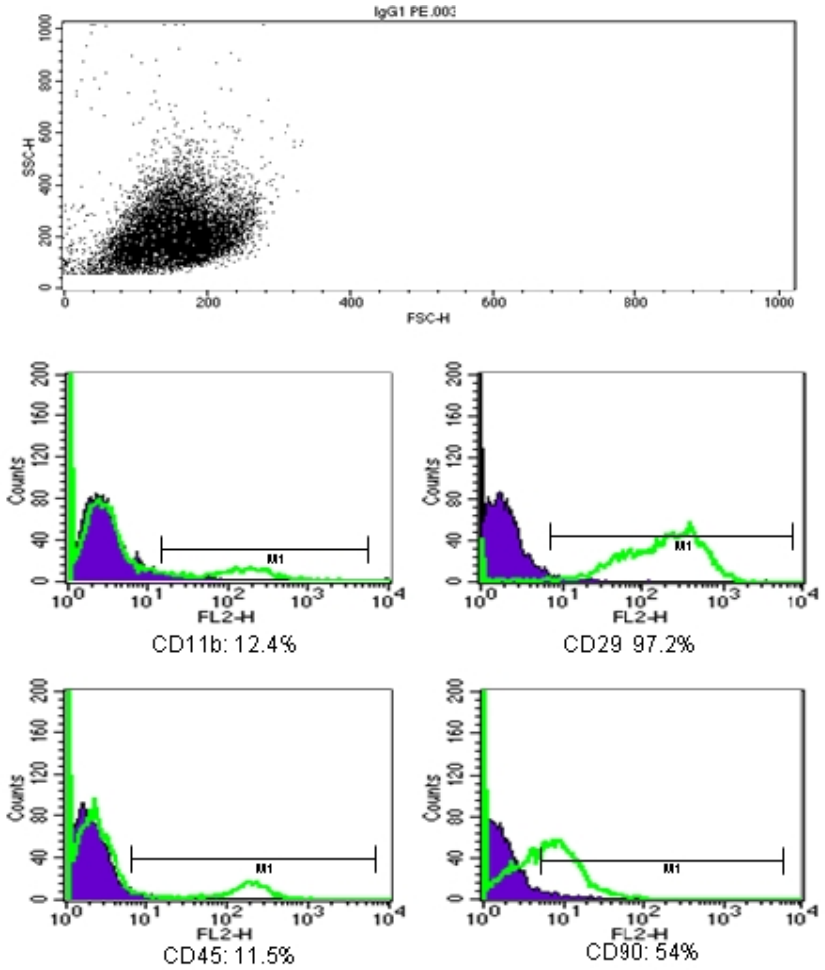


Figure 1: Flow Analysis of Rat Mesenchymal Stem Cells at Passage 2.

Trevigen's rat mesenchymal stem cells (RMSC) were isolated by adherence to plastic from adult male Fisher 344 rat bone marrow. The RMSC were passaged twice to ensure purity of the MSC population.[1,4] By passage 2, the cultures contain less than 1% contaminating hematopoietic cells, as confirmed by flow cytometry (Figure 1). They were positive for CD90 and CD29 and negative for CD11b and CD45 (hematopoietic cell markers). Rat and human mesenchymal stem cells have similar properties *in vitro*, but do not express the same cell surface markers.[1,3]

Trevigen's RMSC are provided as a frozen ampoule containing 1 x 10^6 passage 3 cells. These cells can be maintained in an undifferentiated state when grown in Trevigen's Qualified RMSC Medium (cat# 5000-500-03) supplemented with Qualified RMSC FBS (cat# 5000-050-02) or induced to differentiate into adipogenic (cat# 5010-024-K) or osteogenic (cat# 5011-024-K) phenotype when the appropriate growth medium is supplemented with reagents contained in the Trevigen's differentiation kits. Trevigen's RMSC can undergo 10 doublings without alteration in cell morphology or differentiation potential.

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	Quantity	Storage	Catalog#
Rat Mesenchymal Stem Cells	1 Vial (1 x 10^6 Cells)	Liquid Nitrogen[1]	5000-001-01
Qualified RMSC Medium	500 ml	4°C	5000-500-03
Qualified RMSC FBS	50 ml	-20°C[2]	5000-050-02

[1]Shipped on Dry Ice, immediately thaw for use or for long term storage place in vapor phase of liquid nitrogen.

[2] After initial thaw, FBS should be aliquoted to avoid repeated freeze/thaws. FBS should be thawed at 4°C overnight prior to first use.

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. Cell freezing container

Reagents

1. Cell Harvesting Reagent, trypsin, dispase, etc.
2. Antibiotic Supplement for Media (optional)
3. Sterile PBS (Mg^{2+}, Ca^{2+} free or HBSS)
4. Trypan blue or equivalent viability stain
5. DMSO
6. 70% Ethanol

Disposables

1. Cell culture flask, 25 cm^2, 75 cm^2, or 185 cm^2
2. 15 ml conical tubes
3. 0.22 µm Filter Unit (optional)
4. 1 - 200 µl and 200 - 1000 µl pipette tips
5. 1, 5 and 10 ml serological pipettes
6. gloves
7. 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 RMSC Medium (cat# 5000-500-03):	225 ml
Qualified RMSC FBS (cat# 5000-050-02):	25 ml

Optional: media can be filter sterilized before use
Store media at 4°C for one month
Ensure media are at room temperature or 37°C prior to use

2. 2X Mesenchymal Freeze Medium

For 10 ml of Medium:

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

Mix 1:1 with Growth Medium 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 rat mesenchymal stem cells (RMSC) 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 past 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 medium using a 5 ml pipette
8. Transfer the contents from step 7 to the 15 ml conical tube containing thawed cells dropwise, gently swirling to mix between drops.
9. Add 1 ml of warm medium to 15 ml conical tube containing cells, gently swirling to mix between drops. Total Volume should be about 3 ml
10. Centrifuge 15 ml conical tube at 200 x g for 3 minutes.
11. Remove supernatant gently to avoid disturbing cell pellet
12. Resuspend cell pellet in 1 ml of fresh Mesenchymal Complete Growth medium.
13. Count cells on hemocytometer (per standard protocol)
14. Plate cells at a density of 5.4 x 10^3 cells/cm^2. For a T-75 tissue flask add 4.05 x 10^5 cells in 12-15 ml complete growth medium.
    a. Trevigen recommends Corning® Tissue Culture Treated Plastic
    b. One vial is sufficient to seed two T-75 or one T-185 flask
15. Place Tissue Culture Flask/Dish in 5% CO_2 Tissue Culture Incubator at 37°C
16. Change medium in flasks next day

B. Growing Mesenchymal Stem Cells:

1. Medium Change (the 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 with 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 Rat Mesenchymal Stem Cells

Note: 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.

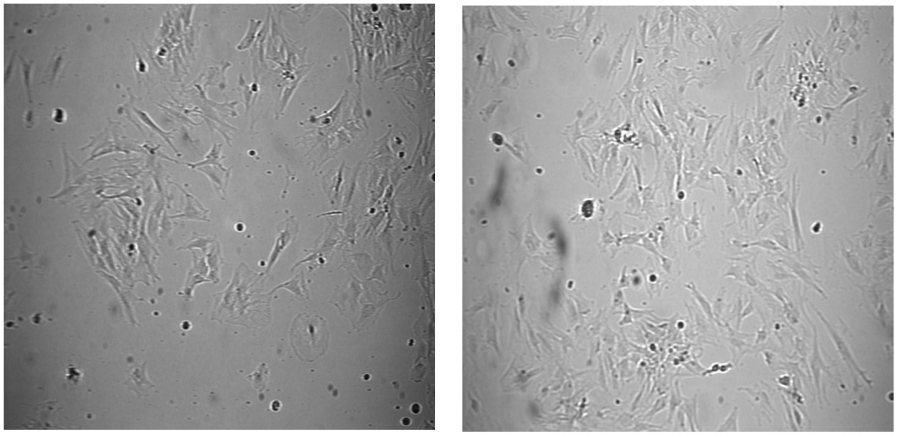
a. Warm Complete Growth Medium and Trypsin solution to 37 °C by placing in 37 °C H_2O bath or in Tissue Culture Incubator
b. Spray down bottles containing Growth Medium, or trypsin with 70% EtOH before placing in Tissue Culture Hood
c. Remove medium from T-75 flask containing Mesenchymal Stem Cells
d. 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 place at 37°C for 3-5 minutes (until cells are no longer attached to plate, should take less than 5 minutes) in Tissue Culture Incubator
g. Add 5 ml of 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.
j. Remove supernatant gently to avoid disturbing cell pellet
k. Resuspend cell pellet in 2 ml of fresh medium
l. Count cells on hemocytometer (per standard protocol)

3. Plating

a. Plate cells at a density of 5.4 x 10^3 cells/cm^2. For a T-75 tissue flask add 4.05 x 10^5 cells in 12-15 ml Complete Growth Medium.

Note: One flask of 70-80% confluent cells should be able to be split into 2-3 T-75 flasks.

Figure 2: 10x Bright Field Images of Rat Mesenchymal Stem Cells



C. Freezing 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. Make 2X Freeze Medium, (see Section V.2) adjust volume according to volume needed (*Will be mixed 1:1 with Growth Medium)*
c. Spray down bottles containing Growth Medium, Trypsin, and the 2X Freeze Medium tube with 70% EtOH before placing in Tissue Culture Hood
d. Remove medium from T-75 flask containing Mesenchymal Stem Cells from incubator
e. Gently wash flask with 5-10 ml of sterile 1X PBS (Ca^{2+} and Mg^{2+} free)
f. Remove PBS
g. Add 3 ml of Trypsin to each flask place at 37 °C for 2-3 minutes (until cells are no longer attached to the plate, which should take less than 5 minutes) in Tissue Culture Incubator
h. Add 5 ml of Complete Growth Medium to flask
i. Transfer cells to 15 ml conical tube
j. Centrifuge 15 ml conical tube at 200 x g for 3 minutes
k. Remove supernatant gently to avoid disturbing cell pellet
    i. Resuspend cell pellet in 2 ml of Complete Growth Medium
    ii. Count cells on hemocytometer (per standard protocol)
    iii. 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, final concentration 0.5 x 10^6 cells/ml). One T-75 flask is sufficient for 1-3 vials of 5 x 10^5 cells per vial.

l. Add equal volume of 2X Freeze Medium to the cells, mix gently
m. Aliquot 1 ml of cells into labeled cryovials
n. Place on ice for 15-30 minutes
o. Transfer to cell freezing container and place in -80°C freezer overnight
p. Transfer to liquid nitrogen freezer for long term storage

Note: Vapor phase is recommended to maintain viability.

VII. References

1. Phinney DG, Prockop DJ. 2007. Concise Review: Mesenchymal Stem/Multipotent Stromal Cells: The State of Transdifferentiation and Models of Tissue Repair-Current Views. *Stem Cells* 25: 2896-2902
2. Kolf CM, Cho E, Tuan RS. 2007. Biology of Adult Mesenchymal Stem Cells: Regulation of Niche, Self-Renewal and Differentiation. *Arthritis Research and Therapy* 9:204-214
3. Javazon EH, Colter DC, Schwarz EJ, Prockop DJ. 2001. Rat Marrow Stromal Cells are More Sensative to Plating Density and Expand More Rapidly from Single-Cell-Derived Colonies than Human Marrow Stromal Cells. *Stem Cells* 19:219-225
4. Li Yi, McIntosh K, Chen J, Zhang C, Gao, Q, Borneman J, Ragniski K, Mitchell J, Shen L, Zhang J, Lu D, Chopp M. 2006. Allogeneic bone marrow stromal cells promote glial-axonal remodeling without immuno-logic sensitization after stroke in rats. *Experimental Neurology* 198:313-25

VIII. Troubleshooting

PROBLEM	CAUSE	ACTION
Poor Cell Recovery from flask (for cell growth)	Cell seeding density too high	Passage cells at lower confluency
Poor Viability from initial freeze	Too rough in thawing of cells	Ensure medium is added slowly to reequillibrate the RMSC 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 and fresh medium was prewarmed to 37°C
Poor proliferation	Fetal Bovine Serum not optimized for support in RMSC growth	Use MSC-Qualified FBS from Trevigen
	Media not optimized for support in RMSC growth	Use MSC-Qualified Media from Trevigen
	Tissue Culture Labware not ideal for RMSC	Used 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 manufactures instructions
	No gas exchange is allowed by flask	Ensure cap is loosened to allow air gas or use vented flask
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 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)
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

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.

Differentiation:

Catalog #	Description	Size
5000-001-01	Cultrex® Rat Mesenchymal Stem Cells	1 vial
5000-001-R	Cultrex® Rat Mesenchymal Stem Cell Replenisher Kit	1 kit
5010-024-K	Cultrex® RMSC Adipogenic Differentiation Kit	24 samples
5011-024-K	Cultrex® RMSC 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
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
4870-500	10X PBS (Ca^{2+}, Mg^{2+} free)	500 ml
5000-050-02	Cultrex® Qualified RMSC FBS	50 ml
5000-500-03	Cultrex® Qualified RMSC Medium	500 ml
3400-010-01	Cultrex® Mouse Laminin I	1 mg
3440-100-01	Cultrex® Rat Collagen I	100 mg
3442-050-01	Cultrex® Bovine Collagen I	50 mg
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
3432-005-02	Cultrex® BME no phenol red, PathClear®	5 ml
3433-005-02	Cultrex® BME no phenol red, reduced growth factor PathClear®	5 ml
3430-005-01	Cultrex® BME with Phenol Red	5 ml
3432-005-01	Cultrex® BME; no Phenol Red	5 ml
3431-005-01	Cultrex® BME with Phenol Red; Reduced Growth Factors	5 ml
3433-005-01	Cultrex® BME no Phenol Red; Reduced Growth Factors	5 ml
3416-001-01	Cultrex® Bovine Fibronectin	1 mg
3420-001-01	Cultrex® Human Fibronectin, PathClear®	1 mg

Catalog#	Description	Size
3417-001-01	Cultrex® Bovine Vitronectin	50 µg
3421-001-01	Cultrex® Human Vitronectin, PathClear®	50 µg
3438-100-01	Cultrex® Poly-L-Lysine	100 ml
3439-001-01	Cultrex® Poly-D-Lysine	100 ml

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