Human Placental Mesenchymal Stem Cell (MSC) Care Manual

INSTRUCTION MANUAL ZBM0097.02

SHIPPING CONDITIONS

Human Placental MSC, Cryopreserved

Cells are shipped using dry ice or dry vapor shipper. Orders are delivered via Federal Express or DHL courier. Alternate couriers are available All US and Canada orders are shipped via Federal Express Priority service and are usually received the within 1-2 days. International orders are usually received in 2-4 days. If your expected transit time will exceed 3 days we strongly suggest the use of a dry vapor shipper for transportation of all cell products.

STORAGE CONDITIONS

Media: +4°C Storage: 30 days from ship date
-20°C Storage: 6 months from ship date

Cryopreserved Cells: Cells are to be stored in vapor phase nitrogen (-150ºC to -190ºC)

IMMEDIATELY UPON RECEIPT. Any other storage negates the warranty.

All Zen-Bio Inc products are for research use only. Not approved for human or veterinary use or for use in diagnostic or clinical procedures.

ORDERING INFORMATION AND TECHNICAL SERVICES

ZenBio, Inc.
3200 East NC-54 Suite 100
PO Box 13888
Research Triangle Park, NC 27709
U.S.A.

Telephone	(919) 547-0692
Facsimile (FAX)	(919) 547-0693
Toll free (continental US only)	**1-866-ADIPOSE 1-(866)-234-7673**
Electronic mail (e-mail)	information@zenbio.com
World Wide Web	http://www.zenbio.com

THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

PLMSC-F	CRYOPRESERVED HUMAN PLACENTAL MESENCHYMAL STEM CELLS

PRECAUTIONS

This product is for basic research use only. *It is not intended for human, veterinary, or in vitro diagnostic use*. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. **Always wear gloves and work behind a protective screen when handling primary human cells.** All media, supplements, and tissue culture ware used in this protocol should be sterile.

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, expressed or implied, including without limitation implied warranties of merchantability or fitness for a particular purpose, are provided by Zen-Bio, Inc. Zen-Bio, Inc. shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

Zen-Bio, Inc. warrants its cells only if Zen-Bio media are used, our shipping recommendations are followed and the recommended protocols are followed without amendment or substitution. Human Placenta Derived Mesenchymal Stem Cells performance also depends greatly on the use of suitable storage of the cells, appropriate media, reagents, and sterile plastic wear. If these parameters are not carefully observed cell responsiveness in assays may be lower than expected.

Contact ZenBio, Inc. within no more than 24 hours after receipt of products for all claims regarding shipment damage, incorrect ordering or other delivery issues. Delivery claims received after 7 days of receipt of products are not subject to replacement or refund.

INTRODUCTION

Human Mesenchymal Stem Cells from Zen-Bio are obtained from donor tissue from consented individuals in the United States. Each donor has confirmed documentation on file allowing for research use of any non-transplantable organs or tissues. .

Due to its pluripotency potential, immunomodulatory properties, and ability to repair tissue, mesenchymal stem cells are an attractive source for research in regenerative medicine research. The cells are assessed for viability and characterized by screening the cells for phenotypic surface markers. The purity of the cells is verified by flow cytometry for the markers, CD73, CD105, CD90, CD34, CD45, CD14, CD19, HLA-DR, and HLA-ABC, reported as a percentage of the population.

Each vial contains a minimum of 500,000 cells/vial.

Cultures from whole placental digests contain multipotent mesenchymal stem cells (MSCs) and are defined by the International Society for Cellular Therapy (ISCT) as meeting the following set of minimal quality criteria:

1. Viability upon thawing at ≥85%
2. Adherence to tissue culture plastic-ware during growth in standard culture conditions.
3. A cell surface marker profile comprising cell surface markers:
    a. POSITIVE for markers
        i. CD105
        ii. CD73
        iii. CD90
    b. NEGATIVE for markers:
        i. CD45
        ii. CD34
        iii. CD14
        iv. CD11b
        v. CD79a
        vi. CD19
        vii. HLA-DR
4. Tri-lineage differentiation potential along osteoblast, adipocyte, or chondrocyte developmental pathways.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

Cryopreserved Human Mesenchymal Cells

- Cat # PLMSC-F
- Frozen vial containing ≥500,000 viable cells

Store in vapor phase liquid nitrogen immediately upon arrival.

MEDIA COMPOSTIONS

Human Placental MSC Growth Medium (catalog MSCG-1 #)	**Storage and Expiration Date**
DMEM, (4.5g/L D-Glucose) Fetal Bovine Serum (FBS) Penicillin Streptomycin Amphotericin	• If placed at 4°C upon arrival, the media is stable for 30 days. • If stored at -20°C upon arrival, the media is stable for **6 months.** The media will expire 30 days after the thaw date.
Cryopreservation Medium (catalog# FM-1-100)	**Storage**
DMSO Fetal bovine serum (FBS)	• Store: -20°C upon arrival

Thawing and Plating Placental MSC

Please note: Primary cells can be very sensitive to brands of cultureware. Zen-Bio does not currently recommend the use of Corning Falcon or Sarstedt brand plates or flasks. Our scientists are using Nunc, Corning Costar, or Greiner Bio-One Cellstar tissue culture treated plates and flasks. Please contact us if you have any questions.

1. Remove cells from liquid nitrogen and place immediately into a 37°C water bath with agitation. Be careful not to submerge the cap of the vial into water. Do not leave the vials in water bath after most of the content has thawed. Rinse the vials with 70% ethanol before taking them to the culture hood.
2. Upon the thawing, add the cells to a sterile conical bottom centrifuge tube, containing 10 ml of Human Placental MSC Growth Medium
3. Centrifuge at 280 x g, 20°C, 5 minutes. Aspirate the medium and resuspend cells in a volume of Human Placental MSC Growth Medium appropriate for counting the cells. Count using a hemocytometer.

4. Place approximately 6.7 X 10^5 cells in T-75 culture flasks using Human Placental MSC Growth Medium.
5. Feed cells fresh Placental MSC Growth medium every 2-3 days.
6. Incubate cells at 37°C and 5% CO_2 until they are 85-90% confluent (in about 4-5 days).
7. Aspirate medium and wash MSC 4-5 times using sterile Phosphate Buffered Saline without calcium or magnesium (PBS) to remove all traces of serum (until there is no foaming of the medium).
8. Remove the PBS and release the cells from the flask bottom by adding 5 mL/T-75 flask (or 10 ml/T-225 flask) of 0.25% trypsin/ 2.21mM EDTA solution.
9. Allow cells to trypsinize for 5 minutes at 37°C. Use your hand to gently tap the flask to loosen the cells.
10. Neutralize the trypsin using 7 ml Human Placental MSC Growth Medium per T-75 flask (or 21 ml per T-225 flask). Check the flask under a microscope to ensure all cells are free of the flask bottom.
11. Count the cells and plate in desired format. Ensure cells are evenly suspended when plating large numbers of plates or flasks.
12. Do not agitate plates and flasks after plating. Place in a humidified incubator at 37°C and 5% CO_2, making sure the surface is level for even cell distribution.

Passaging of Human Placental MSC_____

1. Aspirate the medium from the culture vessels containing cells
2. Wash cells by adding appropriate amount of PBS and gently swirl the culture vessel
3. Aspirate PBS from each well/flask.
4. Add appropriate amount of 0.25% Trypsin/EDTA per culture vessel.
5. Incubate cells with Trypsin 3-8 minutes, wash cells, and collect the cells into an appropriate size centrifuge tube with FBS-containing culture medium that is least 10% of the collected volume to neutralize the cells.
6. Centrifuge cells to pellet and resuspend the pellet with culture medium
7. Count and plate at seeding density of 2,000-4,000 cells/cm^2
8. Add resuspend cells to appropriate amount with medium and plate as per culture vessel (5ml/T25 flask, 13ml/T75 flask, 30ml/T175 flask, 100ml/T500mm flask or 1500ml/Cell Factory)
9. Return culture vessel to a humidified 37ºC incubator in 5% CO_2.
10. ***OPTIONAL* – Cryopreserve cells after counting.**
    a. Centrifuge at 280 x g, 20°C, 5 minutes.
    b. Suspend in cold MSC Growth Medium (Cat# MSCG-1) at a concentration of 500,000 cells/ml. Do not exceed a 6:1 ratio of cells (per million): volume freeze medium (per ml).
    c. Remember to account for the volume of the cell pellet before adding the volume of freeze medium necessary for cell suspension.
    d. If using a controlled-rate freezer:
        i. Freeze by reducing the temperature 1°C per minute until the temperature reaches -80°C.

e. If using a cell cryopreservation container, prepare according to the manufacturer's instructions
f. For best results we recommend transferring the vials to the vapor phase of a liquid nitrogen storage facility as soon as possible after the cells have reached -80°C.

FREQUENTLY ASKED QUESTIONS

1. **Can I passage these cells and what is the maximum passage?**
   Yes. We have achieved a maximum of passage 8. The current passage of each lot is listed on the vial label and noted in the certificate of analysis.
2. **What is the average doubling time of these cells?** Average doubling time varies for each lot but ranges from 24-36 hours.
3. **Are antibiotics included in the medium?**
   Yes. Penicillin, Streptomycin, Amphotericin B.

PATHOGEN TESTING

Samples from each donor are tested and found non-reactive to viral DNA from HIV and hepatitis B, viral RNA from Hepatitis C and CMV and RPR test for syphilis using US Food and Drug Administration (FDA) licensed tests. However, no known test can offer complete assurance that these viruses are not present. Since we cannot test all pathogens, always treat the culture as a potentially infectious reagent. We recommend using the US Centers for Disease Control (CDC) Universal Precautions for prevention of blood-borne pathogens as a minimum guideline for standards of practice at Biosafety Level 1 or higher.