Human Pericyte Care Manual

INSTRUCTION MANUAL ZBM0084.07

SHIPPING CONDITIONS

Human Adult Pericytes, Cryopreserved

Orders are delivered via Federal Express courier. All USA and Canada orders are shipped via Federal Express Priority service and are usually received the next day. Non North American International orders are usually received in 2-4 days. Primary human cells can be sensitive to extended times at dry ice temperatures. If your transit time will exceed 3 days, please inquire about dry vapor shipper options. Please inquire if alternate couriers are needed.

All orders should be processed immediately upon shipment receipt.

STORAGE CONDITIONS

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

Cells: Store in vapor phase nitrogen (-150°C to -190°C) IMMEDIATELY UPON RECEIPT.

Any other storage negates the warranty.

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

THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

PER-F	HUMAN PERICYTES, PLACENTA DERIVED, CRYOPRESERVED, (500,000 VIABLE CELLS/VIAL)

Contents

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 the performance of cells only if Zen-Bio media are used and the recommended storage conditions and protocols are followed without amendment or substitution. Zen-Bio, Inc. cryopreserved cells are assured to be viable when stored as recommended and thawed according to Zen-Bio protocols and using the recommended protocol.

Contact Zen-Bio, 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.

PRECAUTIONS

This product is for 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.

Human pericyte viability depends greatly on the use of suitable media, reagents, and sterile plastic wear. If these parameters are not carefully observed, cell growth may be slower than expected.

INTRODUCTION

Cryopreserved human placental vasculature-derived pericytes are shipped using dry ice or dry vapor shipper (if transit time exceeds 3 days) and should be stored in vapor phase liquid nitrogen **immediately** upon arrival.

Human pericytes are multipotent mesenchymal-like cells found in association with small blood vessel walls. They are important for angiogenesis, the structural integrity of the microvasculature, and blood flow regulation. However, they can also develop into malignant tumors.

Human pericytes contribute to tissue repair. They differentiate into adipocytes during fat tissue injury, into chondroblasts and bone after bone injury, and into myoblasts in a model for muscle dystrophy. Human pericytes have demonstrated the ability to differentiate into fibroblasts and phagocytes (macrophages). Zen-Bio offers pericytes obtained from the placentas of healthy consented adult volunteer donors. Each vial contains 500,000 viable cells/vial.

Human pericytes are obtained from the placenta of a healthy competent consented adult volunteer donor in the United States who has signed an Institutional Review Board (IRB) validated donor consent form that specifically lists both the intended uses for the donation for non-clinical research and confirms the procedures for processing the samples are Standard Operating Procedure (SOP) managed protocols in compliance with ethical regulations.

QUALITY CONTROL

Quality control tests are performed for each lot of human pericyte cells. The cells are characterized by their surface markers via flow cytometry. Population distributions expressed as percentage positive are presented on the certificate of analysis for each lot of cells. The purity of the cells is verified by flow cytometry for the pericyte cell surface markers chondroitin sulfate proteoglycan 4 (NG2), platelet derived growth factor receptor (PDGFr), and CD13. Data are reported as a percentage (%) of the population.

These are phenotypic markers currently used to identify human pericytes. These cells have a guaranteed purity of 80% and a viability of 90%. Each vial contains 500,000 viable cells.

CATALOG ITEMS

- **Pericyte Cryopreservation Medium**
  - Cat # PER-100
  - Store -20°C
- **Pericyte Growth Medium**
  - Cat # PER-1
  - Store according to label
- **Cryopreserved Human Pericytes**
  - Cat # PER-F
  - Cryopreserved vial containing 500,000 viable human pericytes per vial **(store in vapor phase liquid nitrogen IMMEDIATELY upon receipt)** *any other storage negates the warranty*

MEDIA COMPOSITIONS

Pericyte Growth Medium (Cat# PER-1)	**Storage and Expiration Date**
Medium 199 (with Earle's Salts and L-glutamine) Fetal Bovine Serum (FBS; USA Origin) Glycine L-Alanine L-Asparagine L-Aspartic Acid L-Glutamic Acid L-Proline L-Serine Penicillin Streptomycin Amphotericin B	• If stored at 4°C upon arrival, the media is stable until the expiration date on the bottle. • If stored at -20°C upon arrival, the media is stable for 6 months. *The media will expire 30 days after the thaw date.* • Medium is provided ready to use and prepared fresh prior to shipment.

Pericyte Cryopreservation Medium (Cat# PER-100)	**Storage and Expiration Date**
Fetal Bovine Serum (FBS; USA Origin) *Heat Inactivated* Dimethyl Sulfoxide (DMSO)	• Store at -20°C upon arrival until ready for use or the expiration date on bottle. Cryopreservation medium has an expiration date 1 year from the manufacture date when stored frozen. • The media will expire 45 days after the thaw date.

PLATING AND EXPANSION PROCEDURES

THAWING AND CULTURING HUMAN PERICYTES (Catalog # PER-F)

Please note: Primary human pericytes require use of sterile tissue culture treated culture ware. No extracellular matrix coatings are required.

1. Pre-warm Pericyte Growth Medium (cat # PER-1) to 37°C.

2. 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 the water bath after most of the content has thawed, no longer than 1 minute. Rinse the vials with 70% ethanol before taking them into the culture hood.

3. Upon thawing, add the cells to a sterile conical bottom centrifuge tube, containing 10 mL warm PER-1.

4. Centrifuge at 220 x *g* / 20°C for 3 minutes. Aspirate the supernatant. DO NOT ASPIRATE ANY OF THE CELL PELLET. Resuspend in a small amount of PER-1 appropriate for counting, and count using a hemocytometer. The plating density of human pericytes is 3,000-4,000 cells/cm^2.

5. Resuspend cells in the appropriate volume of PER-1 for plating and transfer the cell suspension to designated cell culture vessel. Do not agitate the vessel after the cells have been plated.

6. Place vessel in an incubator (37°C, 5% CO_2) for cell attachment, ensuring a level surface area. Replace medium after 16-24 hours. Harvest or subculture cells once they have reached 70-90% confluency.

OPTIONAL – HUMAN PERICYTE SUBCULTURE

Please note: Human pericytes should not be expanded past passage 4.

1. Pre-warm all reagents and Pericyte Growth Medium (cat # PER-1) to 37ºC.

2. Carefully aspirate medium from cell culture vessel without disturbing attached cells. Wash vessel surface 2 times with sterile Phosphate Buffered Saline without Calcium or Magnesium (cat # DPBS-1000), using approximately 100 µL/cm^2.

3. Carefully aspirate DPBS-1000 from culture vessel and add 0.25% trypsin/ 2.21 mM EDTA solution (cat # TRP-100), using 30 µL/cm^2. Examine cells under microscope and once they begin detaching, gently tap the side of the vessel to loosen any remaining attached cells.

4. Once the cells have detached, neutralize the trypsin using and equal volume of 0.5 mg/mL soybean trypsin inhibitor. Carefully transfer the cell suspension to a sterile centrifuge tube. Centrifuge at 220 x *g* / 20°C for 3 minutes.

5. Aspirate the supernatant. DO NOT ASPIRATE ANY OF THE CELL PELLET. Resuspend in a small amount of PER-1 appropriate for counting, and count using a hemocytometer. The plating density of human pericytes is 3,000-4,000 cells/cm^2.

6. Resuspend the cell pellet into an appropriate amount of PER-1 for plating. Ensure cells are evenly suspended when plating large numbers of plates or flasks. Do not agitate the plates or flasks after plating. Place in a humidified incubator (37°C, 5% CO_2), making sure the surface is level for even cell distribution.

7. Replace the medium 16-24 hours after plating and then every 2-3 days. Once they have reached 70-90% confluency they should be subcultured further or harvested and cryopreserved.

CRYOPRESERVATION PROCEDURE

1. Human pericytes should be sub confluent (less than 90% confluent) upon harvest for expansion or cryopreservation.

2. Aspirate medium and wash cells 4-5 times using sterile Phosphate Buffered Saline without Calcium or Magnesium (cat # DPBS-1000) to remove all traces of serum (until there is no foaming of the medium).

3. Remove the DPBS-1000 and release the cells from the cultureware bottom by adding 30 µL 0.25% trypsin/ 2.21 mM EDTA solution (cat # TRP-100) per cm^2 cultureware surface area (2.25 mL trypsin for T-75 flask).

4. Incubate cells with trypsin solution for 5 minutes at 37°C, monitoring cells under the microscope to ensure they detach.

5. Neutralize the trypsin using 100 µL Pericyte Growth Medium (cat# PER-1) per cm^2 cultureware surface area (7.5 mL PER-1 for T-75 flask). Check under a microscope to ensure all cells are liberated.

6. Transfer the cell suspension to a sterile centrifuge tube. Centrifuge at 220 x *g*/ 20°C for 3 minutes. Aspirate the supernatant, ensuring the cell pellet is not disturbed, and resuspend cells in a volume of PER-1 appropriate for counting the cells. Count using a hemocytometer.

7. Centrifuge at 220 x *g*/ 20°C for 3 minutes. Suspend in cold cryopreservation medium at a concentration of 1 million cells/mL. Do not exceed a 6:1 ratio of cells (per million) to volume cryopreservation medium (per mL). Remember to account for the volume of the cell pellet before adding the volume of cryopreservation medium necessary for cell suspension.

8. If using a controlled-rate freezer: Freeze by reducing the temperature 1°C per minute until the temperature reaches -80° C. If using a cell cryopreservation container, prepare according to the manufacturer's instructions.

9. 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

Can I passage the cells?

All cells are shipped at passage 2 or 3 after establishing a primary culture. We guarantee performance up to passage 4 when our media and protocols are used without amendment or substitution.

How fast do the cells replicate?

The average doubling time is 48-72 hours. However, keep in mind that the replication rate for human pericytes varies from donor to donor.

Should antibiotics be included in the medium?

Yes. Antibiotics and anti-fungal agents are always recommended for use with primary cells.

Where are the cells obtained?

The human pericytes are isolated from human placenta obtained from healthy consented adult volunteer donors in the United States.

Do you test for pathogens? Which ones?

Yes. Samples from each donor are tested via PCR and found non-reactive to viral DNA from HIV and hepatitis B and viral RNA from hepatitis C. However, since we cannot test all pathogens, please treat the culture as a potentially infectious agent at Biosafety Level 1 or higher.

PATHOGEN TESTING

Samples from each donor are tested via PCR and found non-reactive to viral DNA from HIV and Hepatitis B and viral RNA from Hepatitis C. 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. Our products are tested and are free from mycoplasma contamination. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. All human based products should be handled at a BSL-1 (Biosafety Level 1) or higher. Always wear gloves and work behind a protective screen when handling primary human cells.