



Human Pericyte Manual

INSTRUCTION MANUAL ZBM0084.05

SHIPPING CONDITIONS

Human Adult Pericyte Cells

All US and Canada orders are shipped via Federal Express Priority service and are usually received the next day. International orders are shipped via FedEx or DHL service using dry ice or a dry vapor shipper if transit time will exceed 3 days. Primary human cells are very sensitive to extended times (> 3 days) transported using dry ice. Please inquire for dry vapor shipper availability if your transit time will exceed 3 days. Cells should always be stored in liquid nitrogen vapor phase immediately upon arrival.

STORAGE CONDITIONS

- ❖ Media: Short Term (30 days from ship date) 4°C 6 months -20°C
- ❖ Cryopreserved cells: Vials of frozen pericytes are to be stored in vapor phase nitrogen (-150°C to -190°C).

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

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THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

PER-F	HUMAN PERICYTES, PLACENTA DERIVED CRYOPRESERVED, 500,000 CELLS/VIAL
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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, recommended protocols and storage conditions are followed without amendment or substitution.

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.

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 cultureware used in this protocol should be sterile.

Human Pericyte cells' viability depends greatly on the use of suitable media, reagents, and sterile plastic ware. If these parameters are not carefully observed cell responsiveness in assays may be lower than expected.

INTRODUCTION

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.

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. 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 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 GLP protocols in compliance with ethical regulations.

Cryopreserved human placental vasculature-derived pericytes are shipped on dry ice or dry vapor shipper (if transit time exceeds 3 days) and should be stored in vapor phase liquid nitrogen immediately upon arrival. There are 500,000 cells/vial.

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 for 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 reported as a percentage (%) of the population.

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

Cryopreserved human placental vasculature-derived pericytes are shipped on dry ice and should be stored in vapor phase liquid nitrogen immediately upon arrival. There are 500,000 cells/vial.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

Note: Zen-Bio recommends that all cells be processed immediately upon receipt and only if its cells are used with Zen-Bio media and the recommended storage and handling protocols are followed without amendment or substitution.

- **Cryopreserved Human Pericytes, Placenta Derived**

- Cat # PER-F

- Frozen vial containing 500,000 viable human pericytes

- (Store in vapor phase liquid nitrogen immediately upon receipt)

MEDIA COMPOSITIONS

Pericyte Growth Medium Cat # PER-1, 500ml	Expiration Date Information
Medium 199 Fetal Bovine Serum (FBS) Earle's salts L-Glutamine L-Alanine L-Asparagine L-Aspartic Acid L-Glutamic Acid Glycine L-Proline L-Serine Penicillin Streptomycin Amphotericin B	<ul style="list-style-type: none"> • If placed at 4°C upon arrival, the media is stable 1 month from the ship date <ul style="list-style-type: none"> ○ the +4°C expiration date listed on the bottle label. • If stored at -20°C upon arrival, it is stable 6 months after the ship date <ul style="list-style-type: none"> ○ the -20°C expiration date listed on the bottle. ○ Upon thawing, add fresh antibiotics at 1% volume when you are ready to use. ○ The media will expire 30 days after the thawing date.
Cryopreservation Medium Cat# FM-1-100, 100ml	Expiration Date Information
DMEM / Ham's F-12 (1:1, v/v) HEPES pH 7.4 Fetal Bovine Serum (FBS) Biotin Pantothenate	<ul style="list-style-type: none"> • Expiration date listed on bottle • Store -20°C

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PLATING CRYOPRESERVED PERICYTES

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.

Upon arrival store the cryopreserved cells in liquid nitrogen or seed them immediately.

1. Remove the cryovial of pericytes from the liquid nitrogen and immediately place it on dry ice (even for short transportation.). Submerge vial in 37°C water bath and shake for 90 seconds.
2. Rinse cryovial with 70% ethanol and wipe with lint-free lab wiper. Open vial under laminar flow hood and resuspend cells in 9ml of warmed Pericyte Growth Medium. Centrifuge cells for 3 minutes at 220 x g.
3. The plating density of Pericytes is 3,000-4,000 cells/cm². Calculate the necessary culture surface area according to the plating density

4. Resuspend centrifuged pericytes in the appropriate volume of Pericyte Growth Medium and transfer the cell suspension to designated cell culture vessel.
5. Place vessel in an incubator (37°C, 5% CO₂) for cell attachment. Replace medium after 16-24 hours. Harvest cells once they have reached 70-90% confluency.

SUBCULTIVATING PERICYTES

1. Pre-warm all reagents and medium to 37°C.
2. Carefully aspirate medium from cell culture vessel. Wash vessel surface 2 times with Hank's Balanced Salt Solution WITHOUT calcium or magnesium (HBSS) solution (100µl/cm²).
3. Carefully aspirate HBSS from culture vessel and add Trypsin/EDTA solution (100µl/cm²). Examine cells under microscope and once they begin detaching, gently tap the side of the vessel to loosen the remaining cells.
4. Neutralize the trypsin using an equal volume of 0.5mg/ml soybean trypsin inhibitor. Carefully aspirate the cell suspension and transfer to a centrifuge tube. Spin down cells for 3 minutes at 220xg.
5. Aspirate medium and resuspend cell pellet in a desired volume of Pericyte Growth Medium and proceed to cell counting.
6. Seed cells at 3,000-4,000 cells/cm². 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 at 37°C and 5% CO₂, 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 or harvested and cryopreserved.

CRYOPRESERVATION PROCEDURE

1. 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 (PBS) to remove all traces of serum (until there is no foaming of the medium).
3. Remove the PBS and release the cells from the cultureware bottom by adding 2 ml/T-75 flask of 0.25% trypsin/ 2.21 mM EDTA solution.
4. Incubate cells with trypsin solution for 5 minutes at 37°C.
5. Neutralize the trypsin using 0.1 ml Pericyte Medium (cat# PER-1) per cm² cultureware surface area (7.5 ml for T-75 flask). Check under a microscope to ensure all cells are liberated.
6. Centrifuge at 280 x g, 20°C, 5 minutes. Aspirate the medium and suspend cells in a volume of PER-1 appropriate for counting the cells. Count using a hemocytometer.
7. Centrifuge at 280 x g, 20°C, 5 minutes. Suspend in cold cryopreservation medium at a concentration of 1X10⁶ cells/ml. Do not exceed a 6:1 ratio of cells (per million): 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 ---

1. Can I passage the cells?
 - a. 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.
2. How fast do the cells replicate?
 - a. The average doubling time is 48-72 hours. However, keep in mind that the replication rate for human pericytes varies from donor to donor.
3. Should antibiotics be included in the medium?
 - a. Yes.
 - b. Antibiotics and anti-fungal agents are always recommended since the cells are primary cells.
4. Where are the cells obtained?
 - a. The human pericytes are isolated from human placenta obtained from healthy consented adult volunteer donors in the United States.
5. Do you test for pathogens? Which ones?
 - a. Yes. Samples from each donor are tested via PCR to confirm non-reactivity for HIV 1 & 2, hepatitis B, hepatitis C and syphilis. However, since we cannot test all pathogens, please treat the culture as a potentially infectious agent at Biosafety Level 1 or higher.

PATHOGEN TESTING ---

Each lot of primary cells is tested via PCR and found non-reactive to viral DNA from HIV 1 & 2, and hepatitis B, and viral RNA from Hepatitis C and 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. 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. Our products are 3rd Party tested and are free from mycoplasma contamination.