Human Pericyte Manual

INSTRUCTION MANUAL ZBM0084.01

SHIPPING CONDITIONS

Human Adult Pericyte Cells

Human Adult Pericyte cells are shipped on dry ice and should be stored in liquid nitrogen immediately upon arrival. Orders are delivered via Federal Express courier.

Must be processed immediately upon shipment receipt.

STORAGE CONDITIONS

Cryopreserved cells: Vials of frozen Human Pericyte Cells are to be stored in liquid or vapor phase nitrogen (-150°C to -190°C).

Medium: Store at $^{+}$4°C. NOTE: Expiration date is 30 days from the ship date.

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.

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.

ORDERING INFORMATION AND TECHNICAL SERVICES

Life Technologies (India) Pvt Ltd.
306, Agarwal City Mall, Road 44, Pitampura, Delhi - 110034 (India)
Tel: +91-11-4220-8000; 4220-8111; 4220-8222 Fax: +91-11-4220-8444, Mobile: +91-98105-21400
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CONTENTS PAGE

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 and the recommended protocols 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.

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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 placenta-derived pericytes produced at Zen-Bio's facility from normal human tissues. Each vial contains 500,000 viable cells.

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 % 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.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

***Note**: Zen-Bio recommends that all cells be processed immediately upon receipt.*

- **Cryopreserved Human Pericytes**
  - Cat # PER-F
    Frozen vial containing 500,000 viable human pericytes
    (Store in vapor phase liquid nitrogen immediately upon receipt)
    50ml PER-1 support medium (per vial)

MEDIUM COMPOSITION

Pericyte Growth Medium (Cat # PER-1, 500ml)

Medium 199
Earle's salts
L-glutamine
L-Alanine
L-Asparagine•H_2O
L-Aspartic Acid
L-Glutamic Acid
Glycine
L-Proline
L-Serine
Fetal Bovine Serum
Penicillin
Streptomycin
Amphotericin B

The medium is prepared fresh prior to shipment.
The expiration date is 30 days from the ship date when stored at 4°C.
Please schedule your orders accordingly.

PLATING CRYOPRESERVED PERICYTES

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 37ºC 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/cm^2. 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 (37C, 5% CO_2) 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 37ºC.
2. Carefully aspirate medium from cell culture vessel. Wash vessel surface 2 times with HBSS solution (100µl/cm^2).
3. Carefully aspirate HBSS from culture vessel and add Trypsin/EDTA solution (100ul/cm^2). 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 and 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/cm^2. 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 37C and 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 or harvested and cryopreserved.

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.

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 in health individuals.

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, HIV-2, HTLV I, HTLV II, syphilis, CMV, hepatitis B and hepatitis C. However, since we cannot test all pathogens, please treat the culture as a potentially infectious agent at Biosafety Level 2 or higher.

PATHOGEN TESTING

Samples from each donor are tested via PCR to confirm non-reactivity for HIV-1, HIV-2, HTLV I, HTLV II, syphilis, CMV, hepatitis B and hepatitis C. However, no known test can offer complete assurance that the cells are pathogen free. 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-2 (Biosafety Level 2) or higher. Always wear gloves and work behind a protective screen when handling primary human cells.