



Human Exosome Care Manual

INSTRUCTION MANUAL ZBM0091.02

SHIPPING CONDITIONS

Human Exosomes

Orders are delivered via Federal Express courier. All US and Canada orders are shipped via Federal Express Priority service and are usually received the next day. International orders are usually received in 3-4 days. Alternate couriers are available for shipment. Please inquire if needed.

Must be processed upon shipment receipt.

STORAGE CONDITIONS

- Human Exosomes are to be stored at -80°C immediately upon arrival.

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. Not for use in humans.

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

1-(919) 547-0692

Facsimile (FAX)

1-(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.zen-bio.com>

THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

EXP-F100	HUMAN PREADIPOCYTE EXOSOMES, > 1 BILLION PARTICLES/VIAL
EXPLMSC-F100	HUMAN PLACENTAL MESENCHYMAL STEM CELL EXOSOMES, > 1 BILLION PARTICLES/VIAL
EXCBS-F100	HUMAN CORD BLOOD SERUM EXOSOMES, > 1 BILLION PARTICLES/VIAL

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.

To comply with U.S. Food and Drug Administration (FDA) regulations, these products are **not for use in Clinical Diagnostic or Therapeutic Procedures.**

By your acceptance of these products, you are acknowledging that these products will be:

1. Treated as potentially contaminated biological specimens even if accompanying serological reports are negative;
2. Handled by establishing or following appropriate safety control procedures to ensure the safety of using these products.

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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INTENDED USE

- **Exosomes can be used for research in multiple areas. Examples are listed below. No, we do not provide protocols for exosome applications.**
- Exosomes contain protein and nucleic acid (RNA), thus making them an attractive vector of paracrine signals delivered by stem cells.
- Exosomes may also be "loaded" or altered with predetermined proteins and nucleic acid to achieve a desired effect.
- Exosomes can be stored as an allogeneic product having the potential for circumventing many of the limitations of viable cells for therapeutic applications in regenerative medicine.
- *In vitro*, exosomes from preadipocytes stimulate cell proliferation in a wound healing model.
- *In vivo*, adipose-graft derived exosomes have been shown to be a promising tool for skin repair and remodeling.

INTRODUCTION

Exosomes are cell-derived vesicles that can be isolated from many biological fluids as well as from conditioned tissue culture media. Exosomes have been shown to be involved in specialized functions, including intercellular signaling. Exosomes were isolated from cultured human placental mesenchymal stem cells, human subcutaneous preadipocyte conditioned medium or human cord blood using Zen-Bio Standard Operating Procedures from consented donors in the United States. Each volunteer adult donor has signed an Institutional Review Board (IRB) or US Food and Drug Administration (FDA) validated 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 legal and ethical regulations.

Zen-Bio, Inc. human preadipocytes and placental mesenchymal stem cells are characterized by their self-renewing capacity and ability to differentiate into chondrocytes, adipocytes, and osteocytes. This makes them attractive starting materials for tissue engineering and regenerative medicine applications. Since few transplanted cells persist *in vivo*, the beneficial effects of cell therapy may lie in the secreted factors being the active component of this treatment. Exosomes, membrane vesicles that are stored intracellularly in endosomal compartments and are secreted when these structures fuse with the cell plasma membrane are a key part of paracrine secretion.

QUALITY CONTROL

Quality control tests are performed for each lot of Human exosome vesicles. The vesicles are characterized by their protein concentration, estimated RNA concentration, particle concentration/mL, and particle size.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **Human Preadipocyte Exosomes, Frozen** > 1 billion particles/vial frozen exosomes
 - Cat # EXP-F100
 - Frozen vial containing > 1 billion particles/vial frozen exosomes (Store at -80°C upon receipt)

- **Human Placental Derived Mesenchymal Stem Cell Exosomes, Frozen** > 1 billion particles/vial frozen exosomes
 - Cat # EXPLMSC-F100
 - Frozen vial containing > 1 billion particles/vial frozen exosomes (Store at -80°C upon receipt)

- **Human Cord Blood Serum Exosomes,** > 1 billion particles/vial frozen exosomes
 - Cat# EXCBS-F100
 - Frozen vial containing > 1 billion particles/vial frozen exosomes (Store at -80°C upon receipt)

Exosomes are provided in Dulbecco's Phosphate Buffered Saline (DPBS) without calcium or magnesium.

THAWING EXOSOMES

UPON THAWING, PARTICULATES MAY BE OBSERVED. THIS IS NORMAL.

IMMEDIATELY PRIOR TO USE:

1. Remove the vial of exosomes from the -80C freezer and place the vial in a 37°C water bath until the contents are completely thawed.
2. Quickly, rinse cryovial with 70% ethanol and wipe with lint-free lab wiper.
3. BRIEFLY, centrifuge at a low speed (< 100g) to spin down the contents of the vial. This ensures the contents are all recovered and located at the bottom of the vial.
4. Once in solution, particulates remain in solution unless re-frozen

FREQUENTLY ASKED QUESTIONS

1	Do all exosome lots have the same volume?	No, they do not. Volume is based on the amount needed to contain 100ug per vial. Volume ranges have been from 150-500uL.
2	Can the exosomes be labeled?	Yes, by using any one of a variety of fluorescent lipophilic cationic indocarbocyanine dyes, and following the manufacturer's instructions.
3	Are there specific exosome proteins which can be detected by western blotting?	Yes. <ul style="list-style-type: none"> - TSG101(a regulator of vesicular trafficking process) - CD9 (a tetraspanin, involved in cell adhesion) - CD63 (may play a role in growth regulation) -CD81 (a tetraspanin; cell-surface protein that mediates signal transduction events)
4	Will exosomes attach to culture plastic ware?	Exosomes CANNOT be directly cultured and expanded as one would for cultured cells.
5	Can the exosomes be re-aliquoted and refrozen?	Yes. Place the aliquot in Phosphate Buffered Saline (PBS; without calcium or magnesium) directly into a -80°C freezer. Do not exceed 2 freeze/thaw cycles in order to maintain the exosomes' ability to stimulate cell proliferation in a wound healing model.
6	Can exosomes be stored at temperatures other than a -80°C?	Although not specifically tested, storage at -20°C should be suitable if a -80°C freezer is not available
7	What happens if my exosomes thaw?	Exosomes can maintain functionality when stored short-term for up to 3 days at 4°C. -80°C is still the preferred long term storage temperature.
8	I see particulates in my exosomes?	This is normal and not cause for concern. Once in solution, the particulates will remain in solution unless re-frozen.
9	What solution is used to store the exosomes?	Exosomes are provided Dulbecco's Phosphate Buffered Saline (DPBS) without calcium or magnesium.

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 Biosafety Level 1 (BLS-1) or higher. Always wear gloves and work behind a protective screen when handling primary human products.