Human Exosome Care Manual

STORAGE CONDITIONS

Human Preadipocyte Exosomes, Frozen
Human Placental Derived Mesenchymal Stem Cell Exosomes, Frozen

- Vials of frozen Human Exosomes are to be stored in at -80ºC.

All 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.

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.

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INTENDED USE

Exosomes can be used for research in multiple areas as listed below and we do not provide protocols.

- 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" with predetermined proteins and nucleic acid to achieve a desired effect.
- Exosomes can be stored as an "off-the-shelf" 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 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 and human preadipocyte conditioned media using Standard Operating Procedures from consented donors.

Human pre adipocytes 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. A key part of paracrine secretion is Exosomes, which are membrane vesicles that are stored intracellularly in endosomal compartments and are secreted when these structures fuse with the cell plasma membrane.

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**
  - Cat # EXP-F100

    Frozen vial containing 100ug frozen exosomes (Store at -80ºC upon receipt)

- **Human Placental Derived Mesenchymal Stem Cell Exosomes, Frozen**
  - Cat # EXPLMSC-F100

    Frozen vial containing 100ug frozen exosomes (Store at -80ºC upon receipt)

EXOSOME CARE/MAINTENANCE

UPON THAWING, PARTICULATES MAY BE OBSERVED

IMMEDIATELY PRIOR TO USE:

- THAW QUICKLY AND COMPLETELY IN A 37ºC WATER BATH
- VORTEX ON HIGH SETTING FOR 1 MIN. (PARTICULATES WILL GO BACK INTO SOLUTION)
- CENTRIFUGE AT LOW SPEED (≤ 300 rpm or ≤ 100 xg) FOR 1 MIN. TO RECOVER COMPLETE FILL VOLUME IN THE TUBE BOTTOM

ONCE IN SOLUTION, PARTICULATES REMAIN IN SOLUTION UNLESS REFROZEN.

FREQUENTLY ASKED QUESTIONS

1. Do all exosome lots have the same volume?
    a. 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?
    a. 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?
    a. Yes, there are: TSG101(a regulator of vesicular trafficking process), CD9 (a tetraspanin, involved in cell adhesion), CD63 (may play a role in growth regulation) CD81(also a tetraspanin; cell-surface proteins that mediate signal transduction events).

4. Will exosomes attached to culture plastic ware?
    a. Although not specifically tested, since their membranes are comprised of both lipid and protein, it may be reasonable to expect that they would just as cells do. However, exosomes cannot be directly cultured and expanded like cells can.

5. Can the exosomes be re-aliquoted and refrozen?
    a. Yes. Just place the aliquot in DPBS directly into a -80ºC freezer. We have tested two freeze/thaw cycles and still observed exosome ability to stimulate cell proliferation in a wound healing model.

6. Can exosomes be stored at temperatures other than a -80ºC ?
    a. We have kept exosomes at 4ºC for up to 5 days (short term) before using in experiments or aliquoting and placing at -80ºC (for longer term storage). Although not specifically tested, storage at -20ºC should be fine if a -80ºC freezer in not available

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.

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