Product Analysis Certificate

PRODUCT	**Exo-Fect™ ExosomeTransfectionKit**
CATALOG #	**EXFT10A-1**
LOT#	**230726-001**
STORAGE	**See Individual Components**
SHELF LIFE	**12 months from date of receipt with proper storage**
SHIPPING	**Blue ice**

PACKAGE CONTENTS

Component	Amount	Storage
Exo-Fect Reagent	1 x 100 µl	+4ºC
Positive Control siRNA (TX-Red)	1 x 200 µl	-20ºC
ExoQuick TC	1 x 2 ml	RT

DESCRIPTION

Exo-Fect is a novel nucleic acid transfer agent that enables the transfection of nucleic acids directly into isolated exosomes. The transfected si/miRNA, mRNA and even plasmid DNA can then be shuttled into target cells via the transfected exosome vesicles. Simply combine isolated exosome with Exo-Fect and the nucleic acid of your choice to generate exosome delivery vehicles. The protocol takes less than an hour and is highly efficient at placing nucleic acids into exosomes for transport.

Per United States OSHA 29CFR1910.1200, Commonwealth of Australia [NOHSC:1005, 1008(1999)] and the latest amendments to the European Union Directives 67/548/EC and 1999/45/EC, the ExoQuick product **does not require a Material Safety Data Sheet(MSDS).** It does not contain more than 1% of a component classified as hazardous and does not contain more than 0.1% of a component classified as carcinogenic. Therefore SBI does not provide a MSDS for Exo-Fect at this time. When working with Exo-Fect or any laboratory chemicals/reagents, SBI recommends using gloves, lab coats and eye protection as standard practice.

PROTOCOL

Exosome sample preparation

Isolate exosomes from serum or media (isolated by ExoQuick, ExoQuick-TC or ultracentrifugation methods), resuspend the exosome pellet in 500 ulof sterile 1x PBS. The recommended exosome protein input should be approximately 50 - 300 ug exosome protein per transfection reaction (about 1x10^6 exosomes).

Protocol for transfecting exosomes:

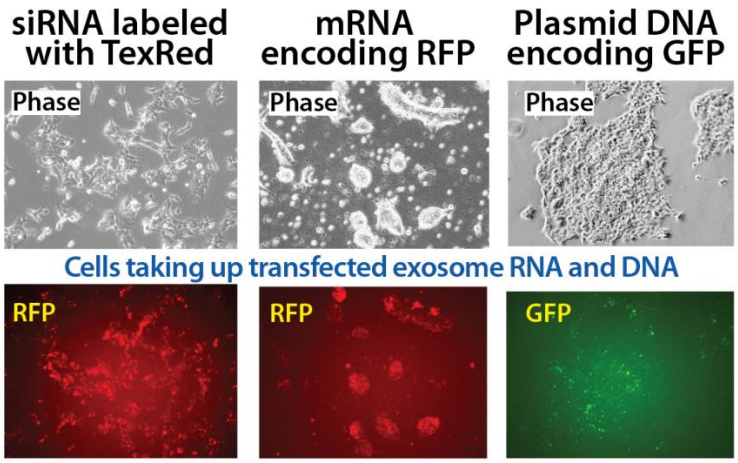
1. In a clean 1.5 ml tube, combine the following:
   10 ul Exo-Fect solution
   +20 ul Nucleic acid (20 pmolsi/miRNA, 1 ug mRNA or 5 ug plasmid DNA)
   70 ul sterile 1x PBS
   50 ul purified exosomes in 1x PBS suspension
   150 ul total transfection reaction
2. Mix the components well by flicking/inversion three times. **Do not vortex.**
3. Incubate the exosome transfection solution at 37°C in a shaker for 10 minutes and then immediately place the tube on ice.
4. To stop reaction, add 30 ul of the **ExoQuick-TC** reagent provided in the kit to the transfected exosome sample suspension and mix by inverting 6 times. **Do not vortex.**
5. Place the transfected exosome sample on ice (or at 4°C) for 30 minutes.
6. Centrifuge the sample for 3 minutes at 13,000-14,000 rpm in a microfuge (top speed).
7. Remove the supernatant and resuspend the transfected exosome pellet in **300 ul1x PBS**.
8. The transfected exosomes are ready to be added to target cells or used in vivo.

Adding Exo-Fect exosomes to cells:

1. Add at least **150 ul of transfected exosomes** to approximately 1x10^5 cells per well in a 6-well culture plate grown in exosome-depleted FBS (such as SBI's Exo-FBS) media. You can scale this ratio up or down depending upon your experimental requirements.
2. Incubate cells for 2 to 24 hours, visualize with fluorescent microscopy using the following excitation/emission guidelines for the fluorophore you are using. For the positive control Texas Red-labeled siRNA: oligo, use standard RFP filter settings on your microscope to visualize the exosomes delivery their cargo to your target cells.

SAMPLE DATA

Exo-Fect kits can be used to transfect intact exosomes with small RNAs (si/miRNAs), longer mRNAs and even plasmid DNA.



Cells were imaged after 24 -48 hours after treatment with transfected exosomes.

The far left panel set shows the transfer of a fluorescently labeled siRNA into exosomes which were then added to target HEK293 cells.

The central panel set shows successful transfection of an mRNA encoding RFP and then having exosomes delivery this mRNA into target HEK293 cells.

The right set of panels shows plasmid DNA that encodes a GFP gene transfected into exosomes with subsequent delivery into HEK293 cells.