Product Analysis Certificate

PRODUCT	**EV Shuttle Transfection Kit**
CATALOG #	**EVS105A-1, EVS110A-1**
STORAGE	**ExoQuick-TC and Exo-Fect reagent at 4°C and the exosome shuttle aliquots at -80°C**
SHELF LIFE	**12 months from date of receipt with proper storage**
SHIPPING	**Dry Ice (-80°C)**

DESCRIPTION

Extracellular vesicles (EVs), including exosomes, are naturally occurring nanocarriers used by cells to transport RNA and protein signals and are central to intercellular communication. These EVs can be transfected (Exo-Fection) with si/miRNAs and mRNAs for loading and subsequent delivery to target cells. The EV shuttle system is especially suited to deliver RNA into hard-to-transfect cells and stem cells.

The EV shuttle Kit contains a novel nucleic acid transfer agent (Exo-Fect) 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 the isolated exosomes provided in the kit with Exo-Fect and the nucleic acid of your choice to generate exosome delivery vehicles. The protocol takes less than an hour and highly efficient at loading nucleic acids into exosomes for transport and delivery.

PACKAGE CONTENTS

Description	catalog#	Size
EV Shuttle Kit Human HEK293 cell exosomes	EVS105A-1	5 Reactions
EV Shuttle Kit Human HEK293 cell exosomes	EVS110A-1	10 Reactions

KIT COMPONENTS

Component	Amount 5 rxn	Amount 10 rxn
Exo-Fect solution	50 ul	100 ul
Frozen exosome aliquots (individual tubes)	5 tubes	10 tubes
ExoQuick-TC precipitation reagent	2 ml	2 ml
Positive control NT siRNA-Texas red label (10 pmol/ul)	50 ul	100 ul

PROTOCOL

Protocol for transfecting exosomes:

1. In a clean 1.5 ml tube, combine the following:
   10 ul Exo-Fect solution
   \+ 10 ul Nucleic acid (20-100 pmol si/miRNA, 1 ug mRNA or 5 ug plasmid DNA)
   80 ul sterile 1x PBS
   50 ul purified exosomes provided in kit
   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 **100 ul 1x PBS**.
8. The transfected exosomes are ready to be added to target cells or used in vivo.

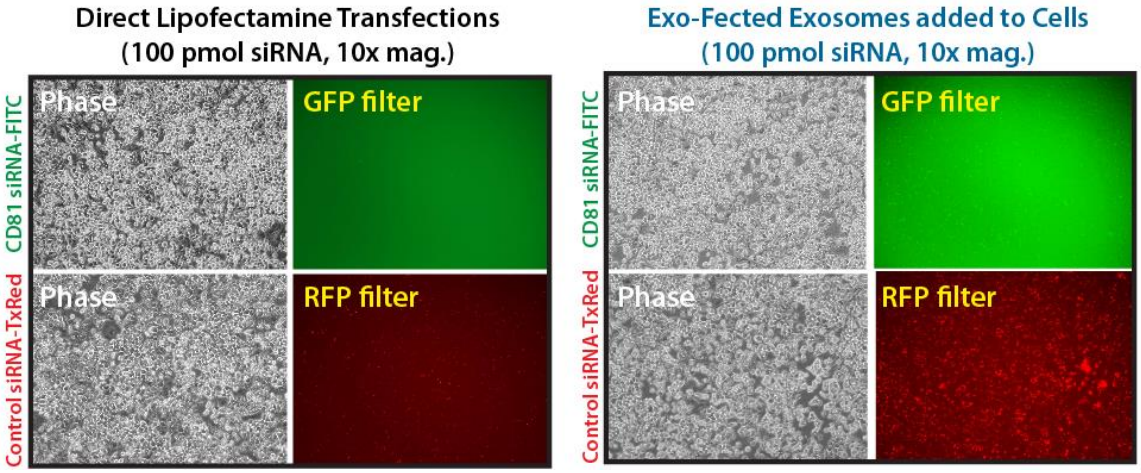
Adding Exo-Fect exosomes to cells:

1. Add at **50 - 100 ul of transfected exosomes** to approximately 2x10^5 cells per well in a 12-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.

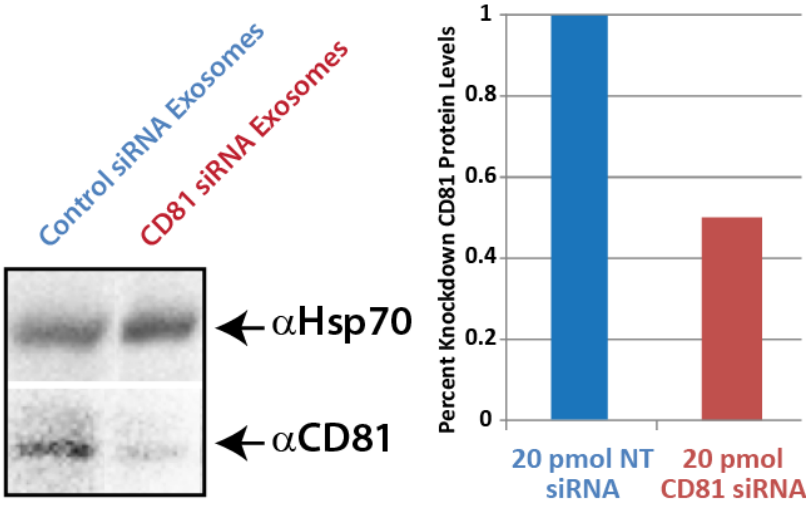
2. Incubate cells for at least 24 hours although cargo delivery can occur in some cases as rapidly as 2 hours. Monitor the delivery by visualizing with fluorescent microscopy using the following excitation/emission guidelines for the fluorophore you are using. For the positive control Texas Red-labeled siRNA: oligo, use standard RFP filter settings on your microscope to visualize the exosomes delivery their cargo to your target cells.

SAMPLE DATA

HEK293 exosomes (human) HEK exosomes were Exo-Fected with 20 pmol of either Control siRNA-TxRed or CD81 siRNA-FITC. The EV shuttles were then added to Mouse macrophage cells (RAWS 264.7, hard-to transfect) in parallel with performing a standard Lipofectamine transfection of the same siRNAs with RAWS 264.7. The data show that human exosome shuttles are taken up by mouse RAWs 264.7 cells at a much higher efficiency than with Lipofectamine transfection protocols.



The RAWS 264.7 cells were harvested and protein lysates prepared for Western blot analysis of the knock down of the CD81 protein in the mouse RAWS 264.7 target cells. The Western blot and subsequent band intensity signal quantitation reveal an over 50% knockdown of the mouse CD81 protein level using EV shuttle delivered siRNA targeting CD81 mRNA in the RAWS 264.7 cells. These data show that as little as 20 pmol CD81 siRNA can be loaded into exosomes and delivered to hard-to-transfect cells to achieve a significant knock down effect. The amount of a given siRNA or miRNA may have to be titrated to find the ideal concentration to achieve the desired knock down/effect for your model system and target mRNA.



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