ExoFectin® sRNA-into-Exosome Kit (Chemical)

Cat. #: P401

Storage: 4°C (Do not freeze)

Shelf Life: 6 months

Application: ExoFectin **sRNA-into-Exosome Kit (chemical)** is for the loading of nucleic acids including miRNAs or siRNA into exosomes isolated by our kits. This product is for research use only.

Product Size: 100 µL ExoFectin Reagent (~ 20 reactions)

Product Description: **ExoFectin sRNA-into-Exosome Kit (chemical)** is a unique blend of polymers designed for the delivery of small RNAs including miRNA and siRNA into exosomes. This transfection reagent provides highly efficient loading abilities of miRNA or siRNA into exosomes, allowing modified exosomes to carry and deliver small RNAs into target cells. This kit provides the following advantages:

» efficient gene delivery
» gentle on treated exosomes
» no small RNA aggregation in exosome

Kit Contents:

	Amount	Storage
ExoFectin Reagent	0.1 mL	4°C
Supplemental buffer	1.0 mL	4°C

Required materials that is not provided in this kit:

- miRNA or siRNA
- Exosome (pure exosome isolated by our PureExo or DiagExo kits is preferred)
- Eppendorf tubes

Positive control (optional): use Alexa Fluor® Red Fluorescent Oligo (Life Technologies, Cat. no. 14750100) to determine transfection efficiency.

Protocol:

1. Prepare / isolate pure exosomes (50-100 µg) using our kits (Cat.#: P100, P101, D100 or D101). If the isolated exosome is in the form of pellets, resuspend the exosome pellets in 10 - 100µl **1× PBS** (depend on the size of exosome pellets, about 10× volume of exosome pellet). Keep the exosome suspension on ice. If start with frozen exosome sample, thaw it at room temperature completely, mix well, and then keep on ice.
   Note: Concentrated exosome will precipitate, pipet up and down to mix well before each use.
2. In tube 1, prepare ExoFectin suspension:
   a) Supplemental buffer **25 µL**
   b) ExoFectin Reagent **5 µL**
   Mix gently
3. In tube 2, prepare siRNA or miRNA suspension:
   a) Supplemental buffer **25 µL**
   b) siRNA (10 µM) **~1 µL** (10 pmol)
   or miRNA (5µM) **~1 µL** (5 pmol)
   Mix gently

4. Add **25 μL** ExoFectin suspension to **25 μL** siRNA/miRNA suspension to make siRNA/miRNA-ExoFectin complex, and mix well by pipet up and down for **10 seconds**.
5. Incubate at room temperature for 5 minutes.
6. Add **50μL** siRNA-ExoFectin complex or miRNA-ExoFectin complex to exosomes (**50-100 μg**).
7. Incubate at **37°C** for **overnight**.
8. Harvest the transfected exosomes using PureExo® Kit (Cat.#: P101). Refer to P101 manual. The exosome is transfected and ready for the downstream experiment.

Reaction summary table

Components used		Steps
Supplemental buffer ExoFectin Reagent	25 μL 5 μL	1) Dilute ExoFectin Reagent in Supplemental buffer
Supplemental buffer siRNA (10 μM) miRNA (5μM)	25 μL 1 μL (10 pmol) 1 μL (5 pmol)	2) Dilute siRNA or miRNA in Supplemental buffer
Diluted ExoFectin solution Diluted siRNA or diluted miRNA	25 μL 25 μL	3) Add 25 μL diluted siRNA or diluted miRNA to 25 μL diluted ExoFectin Reagent (1:1 ratio)
Incubate		4) Incubate for 5 minutes at R.T.
Exosomes	50-100 μg	5) Add 50μL siRNA-ExoFectin complex or 50μL miRNA-ExoFectin complex to exosomes (50-100 μg) 6) Incubate for overnight at 37°C.

7) Harvest the transfected exosomes using PureExo® Kit (Cat.#: P101).

Suggested downstream application of these transfected exosomes:

a. Deliver the transfected exosomes to target cells: resuspend the transfected exosomes in 1× PBS containing 1% BSA. Apply the transfected exosomes to 0.5~1 × 10^4 the cells. Continue culturing the cells for 24 hours, and then harvest the treated cells for downstream assays (eg. measuring target gene expression using real time RT-PCR.)

b. *In vivo* exosomes delivery (Intravenous delivery, such as tail injection, or local injection, such as intramuscular injection of transfected exosomes into animals): resuspend isolated exosome in 5% glucose normal saline and inject to recipient animals. Repeated injection will increase the efficiency of the exosome delivery. It is highly recommended to choose the same strain of recipient animal in agree with the exosomes source to minimize the immune rejection. At various time points after the exosome delivery, measure target gene expression in the tissue of interest using real time RT-PCR or detect target gene expression using imaging methods.