ExoFectin® **sRNA-into-Exosome Kit** (Electro)

Cat. #: P400

Storage: store at **4ºC** **Shelf Life:** 6 months

Application: ExoFectin® **sRNA-into-Exosome Kit** is for the loading of nucleic acids including miRNAs and siRNA into pure exosomes isolated by our kits.

Product Size: 10 reactions

Product Description (This product is for research use only.)

ExoFectin® **sRNA-into-Exosome Kit** is a proprietary formation designed for the delivery of small RNA including miRNA and siRNA into exosome. This kit provides the following advantages:

- high loading efficiency
- gentle on treated exosomes
- easy to use

Components	Content
ExoFectin Solution A	700 µl
ExoFectin Solution B	400 µl
Electroporation cuvettes	10
Sterile transfer pipettes	10

Protocol

1. Start with the pure exosomes isolated by 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 10x volume of exosome pellet). Keep the exosome suspension on ice.

2. Take **2-5µl** exosome suspension to quantify the exosomal protein. The total exosomal protein concentration represents the quantification of exosome.

3. Mix **65 µl** ExoFectin Solution A and **35 µl** ExoFectin Solution B, combine the mixture with exosomes (**10 – 100 µg**) and small RNA (**0.1 – 3 µmol**), pipet up and down to mix well. *Total volume of the mixture should not exceed 120 µl.

4. Carefully, transfer **ExoFectin-exosome-smallRNA** mixture into a cuvette (the mixture covers the bottom of the cuvette avoiding air bubbles). Cover the cuvette with the cap.

5. Insert the cuvette with exosome/small RNA mixture into electroporator cuvette holder. Electroporate the mixture at **400 mV** with the pulse time of **10-15 ms**.

6. Take the cuvette out once the electroporation is completed. Add **500 µl** 1× PBS to the cuvette and use the provided "fine tip transfer pipette" to transfer the mixture gently into a new sterile tube preloaded with **1.5 ml** 1× PBS containing 1% BSA. Now, the exosomes are loaded with small RNA.

7. (Optional) Precipitate the loaded exosomes using PureExo® Exosome Isolation Kit (Cat.#: P100). Refer to P100 manual.

8. Downstream application of these loaded exosomes:

   8.1. Deliver RNA to target cells with loaded exosomes: resuspend the isolated exosomes in 1× PBS containing 1% BSA. Starve target cells for 48 hours or culture target cells with exosome depleted FBS until cells reach 50% confluence. Apply the small RNA loaded exosomes to the cells. Continue culturing the cells for 48 to 72 hours, and then harvest the treated cells to measure target gene expression using real time RT-PCR.

   8.2. *In vivo* RNA delivery (Intravenous delivery, such as tail injection, or local injection, such as intramuscular injection of loaded 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.

Remarks:

The efficiency of electroporation transfection depends on the quality of the exosomes. We do not suggest to start with impure exosome samples prepared by PEG method.

The efficiency of electroporation also depends on the optimal concentration of the exosomes and small RNA. Different RNA and exosome from different source may have different optimal concentration. We suggest customer to test different combinations in the first experiment.

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