Exo-Fect™ siRNA/miRNA Transfection Kit

Cat # EXFT200A-1

User Manual

Storage: Please see individual components

Version 1
4/1/2019

Contents

Product Description

Extracellular vesicles (EVs) released from cells have been an emerging and exciting field of research for basic and translational researchers. They are known to carry nucleic acid cargo such as microRNAs and mRNAs which may play fundamental roles in altering the biology of recipient cells[1]. Taking advantage of their propensity to act as carriers for biological cargo, efforts have been made to engineer EVs to deliver desired microRNAs and other small RNAs for functional studies of EV-mediated nucleic acid delivery[2] as well as therapeutic applications[3].

Efforts to deliver small RNA cargo to EVs have been traditionally done through electroporation (EP)[4]. While relatively robust in delivery efficiency, electroporation approaches often require optimization of voltage settings and timing to achieve consistent loading as well access to specialized. To address these shortcomings, SBI is proud to introduce Exo-Fect™ siRNA/miRNA Transfection Kit (Cat #EXFT200A-1), a first-of-its-kind exosome transfection kit specially designed to load siRNA/miRNAs into EVs. It is based on a proprietary modified Cell-Penetrating Peptide (CPP) technology to complex siRNA/miRNAs and entry into EVs, followed by a depletion method to remove free CPP. This two step method allows for robust loading of EVs with siRNA/miRNA cargo without the need for cumbersome EP methods, freeing up the researcher to focus on the biology, not the underlying technique.

List of Components

Item	Volume/Qty	Storage Temperature
Transfection reagent	80 µl	4^{0}C
Transfection buffer	2 ml	RT
Cy3 transfection control (10 µM)	50 µl	**-20^{0}C**
Clean-up columns	20 columns	4^{0}C
Collection tubes	20 tubes	RT
Column buffer	20 ml	RT

Storage

The Kit is shipped on blue Ice and the components should be stored at recommended temperatures as stated above. Properly stored kits are stable for 12 months from the date received.

General Information

One reaction is defined as a transfection in a well of 6-well plate

Protocol:

Cell seeding:

Cells should be plated ~24 hrs prior to transfection to the optimal density of 60-70%. Change the media to a fresh one before transfection. Reverse-transfection protocol is optional.

EV loading and cleanup:

1. Thaw Cy3 transfection control (or siRNA of your choice) on ice.
2. Prepare loading reaction according to the guidelines in Table 1, the setup for Cy3-labeled control is provided below (for one well of a 6-well plate):

Reagent	Sample reaction (+EVs)	Control reaction (-EVs)
Cy3 transfection Control (10 µM)	2.5 µl	2.5 µl
Transfection reagent	4 µl	4 µl
Transfection Buffer	100 µl	100 µl
Total volume	107 µl	107 µl

3. Incubate for 15 min at Room Temperature (RT) **in dark** if your siRNA is fluorescently labeled.
4. Add isolated EVs (50-300 µg in 100 µl) to the reaction mixtures from step #3. The exact amount of EVs should be optimized for your experiment.

Reagent	Sample reaction(+EVs)	Control reaction (-EVs)
EVs (50-300 µg)	100 µl	-
1xPBS	-	100 µl
Total reaction volume	207 µl	207 µl

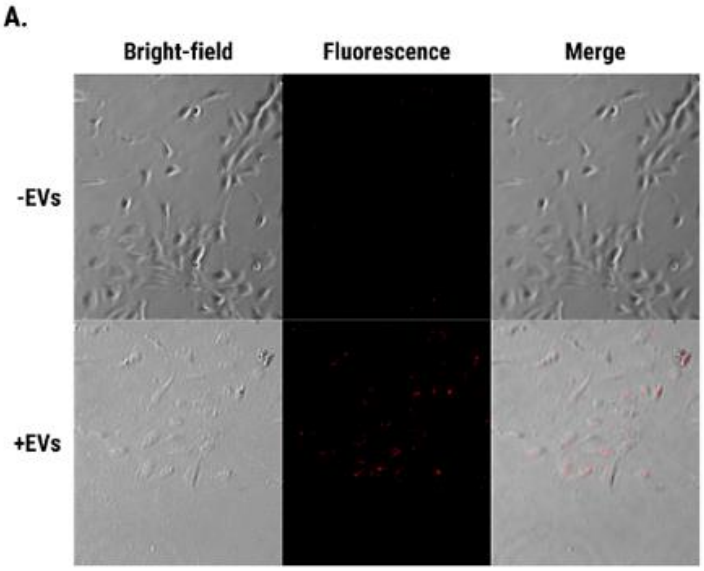
! **NOTE:** We don't recommend using DMEM or any other media during the loading reaction.

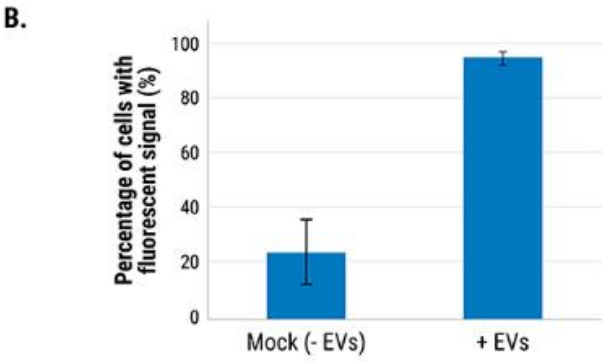
5. Incubate reaction tubes for 1 hr at 37°C in the dark.
6. Take out Clean-up column, loosen screw cap and snap off the bottom closure. Place the column into a collection tube.
   ! **NOTE:** save the bottom closure for steps 13-15.
7. Centrifuge at 1,000 x g for 30 seconds to remove storage buffer.
8. Decant the flow-through and place the column back into the collection tube.
9. To wash the column, remove the cap and apply 500 µl of column buffer on top of the resin and centrifuge at 1,000 x g for 30 seconds. Decant the flow through.
   ! **NOT**E: save the cap for steps 13-15.
10. Repeat step 9 one more time to wash the column.
11. Plug the bottom of the column with the bottom closure.
12. Add the entire content of the sample from step 5 to the resin in the column. Securely place the top cap on the column.
13. Mix at room temperature (RT) on a rotator for 15 minutes.
    ! **CAUTION:** sample will start to elute as soon as the bottom closure is removed.
14. Transfer the column to a 2 ml tube, loosen the screw cap and remove the bottom closure.
15. Centrifuge at 1,000x g for 30 seconds to obtain transfected (loaded) EVs.
16. Discard the column.
17. Apply the samples directly to cells growing in the appropriate medium.
18. Incubate the cells overnight.
19. Next day, wash the cells with room temperature 1xPBS and add fresh media.
20. Image the cells immediately or within 24-48hrs.

Table 1: Guidelines for the ratio between siRNA/Transfection reagents per each culture format

Culture dish	Growth Medium (ml)	Transfection Buffer (µl)	siRNA (nM) final conc. per well	Transfection reagent (µl)	EVs (µg)
24-well	0.5	50	5-100	2	50-300
12-well	0.75	75		3	
6-well	1	100		4	
60 mm	3	300		12	

Example Data and Applications





C.

Cell line	EV Cargo Delivery Efficiency
HeLa	~95%
HEK293	~90 - 95%
HUVEC	~80 - 90%

Figure 1. Exo-Fect siRNA/miRNA Transfection Reagent efficiently loads siRNA into EVs. (A) Cy3-labeled control siRNA were loaded into EVs, delivered to primary HUVECs, and cells imaged 36-48 hours post transfection. Shown are the bright-field images (left panels), fluorescence images (middle panels), and merged images (right panels). The top row of images are cells minus EVs. The bottom row shows cells treated with EVs that were loaded with Cy3-labeled siRNA. EV treatment results in the transfer of Cy3-labeled siRNA to most of the imaged cells (bottom row). The negligible fluorescence seen in the minus EV treated cells (top row) is due to the minute traces of labeled siRNA that escaped the clean-up step. **(B)** Quantitation of the EV cargo delivery efficiency shown in (A). **(C)** EV transfection is highly robust with loaded EVs efficiently transferring cargo to a range of different cell types.

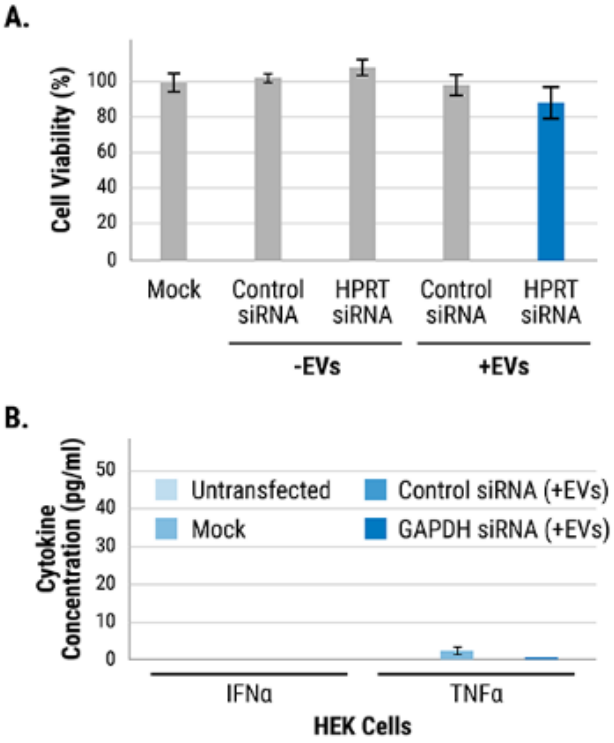


Figure 2. Exo-Fect siRNA/miRNA reagent is non-toxic to cells exposed to treated EVs. (A) HeLa cells exposed to mock preparations, control siRNA preparations, and siRNA-loaded EVs show high viability regardless of treatment conditions. **(B)** Measurement of cytokines also shows the low toxicity of the Exo-Fect siRNA/miRNA reagent. Conditioned medium from cells exposed to untransfected EVs, mock EVs, EVs loaded with control siRNA, or EVs loaded with GAPDH-targeting siRNA all show low levels of cytokines, indicating that all are healthy, non-stressed cells.

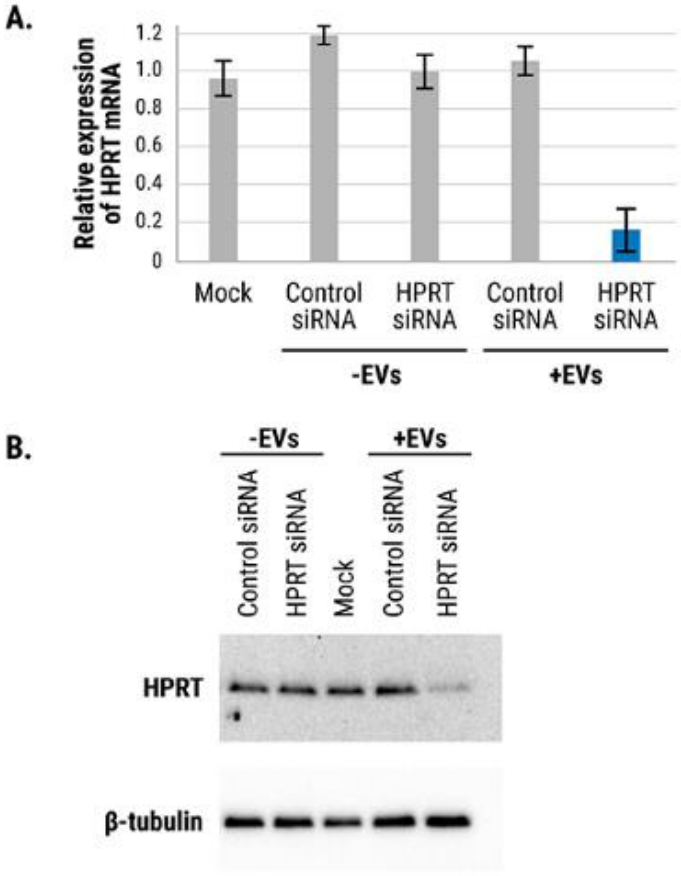


Figure 3. EVs loaded with siRNA using Exo-Fect siRNA/miRNA reagent efficiently deliver functional cargo into recipient cells. (A) Only cells treated with EVs containing HPRT-targeting siRNA show a knock-down in HPRT expression via qPCR. Mock-treated cells and cells exposed to EVs loaded with control siRNA all show normal HPRT expression. (B) The knock-down in HPRT expression is further confirmed at the protein level, as demonstrated via Western blot.

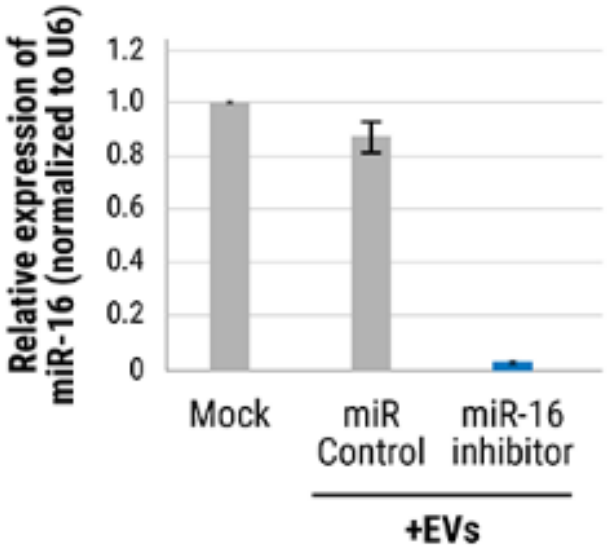


Figure 4. EVs loaded with anti-miRNA using Exo-Fect siRNA/miRNA reagent are functional. EVs loaded with an miR-16 inhibitor (anti-miR 16) using Exo-Fect siRNA/miRNA reagent strongly reduce miR-16 expression in HeLa cells. The expression of miR-16 was assessed by qPCR 24-hours post-transfection using U6 RNA as a reference.

References

1. Valadi H *et al.* Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol.* 2007 Jun;9(6):654-9. Epub 2007 May 7.

2. Pegtel DM, Cosmopoulos K, Thorley-Lawson DA*, et al.* Functional delivery of viral miRNAs via exosomes. *Proc Natl Acad Sci U S A.* 2010;107(14):6328-33.

3. Chen Z *et al.* Therapeutic Potential of Mesenchymal Cell-Derived miRNA-150-5p-Expressing Exosomes in Rheumatoid Arthritis Mediated by the Modulation of MMP14 and VEGF. *J Immunol.* 2018 Oct 15;201(8):2472-2482. doi: 10.4049/jimmunol.1800304. Epub 2018 Sep 17.

4. Alvarez-Erviti, L *et al.* Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol* 29: 341–345

Technical Support

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