Exo-Fect™ siRNA/miRNA Transfection Kit

Cat # EXFT200A-1

User Manual

Storage: Please see individual components

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**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 as 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**

<table>
<thead>
<tr>
<th>Item</th>
<th>Volume/Qty</th>
<th>Storage Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfection reagent</td>
<td>80 µl</td>
<td>4°C</td>
</tr>
<tr>
<td>Transfection buffer</td>
<td>2 ml</td>
<td>RT</td>
</tr>
<tr>
<td>Cy3 transfection control (10 µM)</td>
<td>50 µl</td>
<td>-20°C</td>
</tr>
<tr>
<td>Clean-up columns</td>
<td>20 columns</td>
<td>4°C</td>
</tr>
<tr>
<td>Collection tubes</td>
<td>20 tubes</td>
<td>RT</td>
</tr>
<tr>
<td>Column buffer</td>
<td>20 ml</td>
<td>RT</td>
</tr>
</tbody>
</table>

**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):

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Sample reaction (+EVs)</th>
<th>Control reaction (-EVs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cy3 transfection Control</td>
<td>2.5 µl</td>
<td>2.5 µl</td>
</tr>
<tr>
<td>(10 µM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transfection reagent</td>
<td>4 µl</td>
<td>4 µl</td>
</tr>
<tr>
<td>Transfection Buffer</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>Total volume</td>
<td>107 µl</td>
<td>107 µl</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Sample reaction(+EVs)</th>
<th>Control reaction (-EVs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVs (50-300 µg)</td>
<td>100 µl</td>
<td>-</td>
</tr>
<tr>
<td>1xPBS</td>
<td>-</td>
<td>100 µl</td>
</tr>
<tr>
<td>Total reaction volume</td>
<td>207 µl</td>
<td>207 µl</td>
</tr>
</tbody>
</table>

! **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.
   ! **NOTE:** 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

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

Example Data and Applications

A.

![Bright-field, Fluorescence, Merge](image)

- EVs

+ EVs

B.

![Percentage of cells with fluorescent signal](image)

C.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>EV Cargo Delivery Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa</td>
<td>~95%</td>
</tr>
<tr>
<td>HEK293</td>
<td>~90 - 95%</td>
</tr>
<tr>
<td>HUVEC</td>
<td>~80 - 90%</td>
</tr>
</tbody>
</table>
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.

![A.](image1)

![B.](image2)

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


Technical Support
For more information about SBI products and to download manuals in PDF format, please visit our web site: http://www.systembio.com

For additional information or technical assistance, please call or email us at:

System Biosciences (SBI)
2438 Embarcadero Way
Palo Alto, CA 94303

Phone: (650) 968-2200
Toll-Free: (888) 266-5066
Fax (650) 968-2277

E-mail:
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

Licensing and Warranty Statement
Limited Use License

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The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:
• The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.

• The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.

• This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

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