PRECISION NANOSYSTEMS
Create Transformative Medicines™

siRNA Spark™ Transfection Kit - 5nmol

This protocol provides instructions for the encapsulation of 5 nmol of siRNA into nanoparticles on the NanoAssemblr ™ Spark using various siRNA Spark Transfection Kits. Hepato9™ and Neuro9™ are the specific Spark Transfection Kits that are to be used with this protocol. The manufactured siRNA nanoparticles can be used to knockdown gene expression in cell cultures.

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1. Document Information

About this Document	
Issue Date	22 April, 2016
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Associated Documents	
Document Title	Document Number
Spark User Guide	PNI-MN-NA-003-EXT
Ribogreen Assay	PNI-SOP-NAS9-003-EXT
Ribogreen Quantification Workbook	PNI-SOP-NAS9-003-EXT

2. Kit Equipment, Reagents and Consumables [Provided]

Equipment	Special Instructions
siRNA Spark Cartridge - Qty 1 The sterile pack contains a base referred to as the Cartridge and a cover termed the Cartridge Cap.	Sterile

Kits and Reagents	Special Instructions
siRNA Spark Nanoparticle Mix†* - 100 µL	Sterile; Store at -80 °C
siRNA Spark Formulation Buffer 1* – 200 µL	Sterile; Store at 4 °C
siRNA Spark Formulation Buffer 2* – 1200 µL	Sterile; Store at 4 °C
ApoE – 100 µg	Sterile; Store at -80 °C

† A fluorescently-labeled dye can be supplemented to visualize/quantify nanoparticle uptake in cells. Please contact Precision NanoSystem, Inc. for additional details.
* Each vial has a descriptor (Neuro9 or Hepato9) on the label for use in a specific cell type.

3. Equipment, Reagents and Consumables [Required]

Equipment	Ordering Information
NanoAssemblr Spark†	Precision NanoSystems, Inc.
Micropipettes (10, 20, 200, and 1000 µL)	Eppendorf*
-80 °C Storage	
4 °C Storage	
NanoDrop™ 2000c UV-Vis spectrometer	ThermoFisher Scientific*

Kits, Reagents, and/or consumables	Ordering Information
RNase-free filter pipette tips (10, 20, 200, and 1000 µL)	ThermoFisher Scientific (cat # AM12635, AM12645, AM12650, AM12660)*
RNase-free microcentrifuge tubes (1.5 mL)	ThermoFisher Scientific (cat # AM12400)*
siRNA (lyophilized)	Acquired by Customer

* Equipment, reagents, and consumables referenced are recommendations. Substitutes or alternatives can be used but may require additional optimization. Contact Precision NanoSystems for further information.
† Kit is calibrated for use with the NanoAssemblr Spark and cannot be used with any other instrument.

4. Equipment, Reagents and Consumables [Optional]

Equipment	Ordering Information
Synergy H1 Multi-Mode Reader	BioTek*

Kits, Reagents, and/or consumables	Ordering Information
Triton™ X-100	Sigma (cat # T8787)*
Quant-iT™ RiboGreen® RNA Assay Kit	ThermoFisher Scientific (cat # R11490)*
96-well Plate	Corning (cat # 3370)

* Equipment, reagents, and consumables may be substituted with alternative options along with proper optimizations.

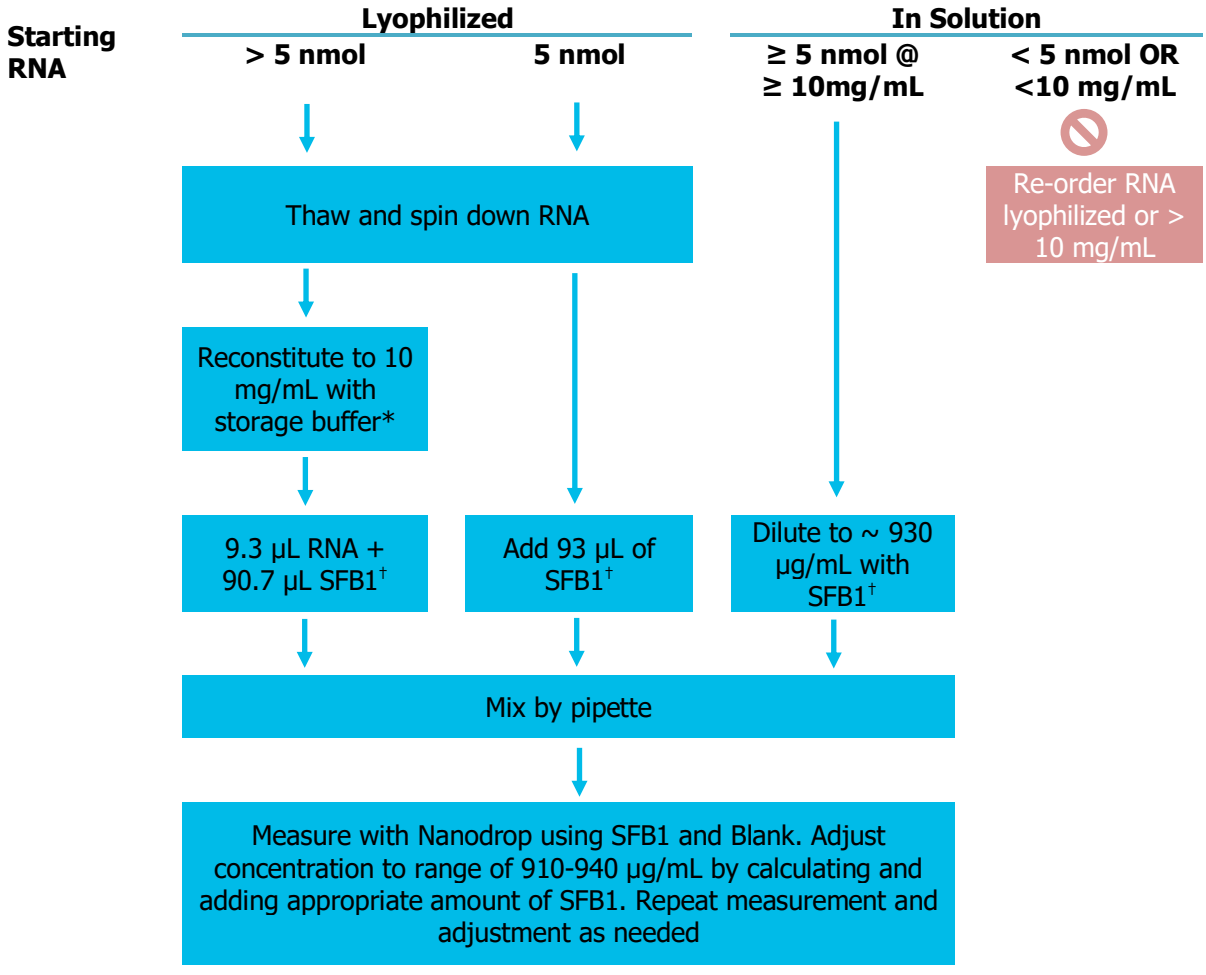
5. Protocol Instructions

Notes:

1. ***It is preferable to start with lyophilized siRNA. For RNA in solution, a concentration of at least 10 mg/mL is required.***
2. ***Please maintain sterile conditions. Conduct all steps in this section in a sterile biosafety cabinet.***

5.1. Preparation of siRNA

Depending on the starting format of your siRNA, follow the appropriate preparation instructions below:



* Use storage buffer recommended by RNA provider and promptly store unused/remaining RNA.
† SFB1 = siRNA Spark Formulation Buffer 1

5.2. Manufacturing siRNA Nanoparticles

Note: Refer to Figure 1 for an illustrated workflow.

1. Place the NanoAssemblr Spark inside the biosafety cabinet and connect the instrument to the power supply by following the instructions provided in the Spark User Guide.
2. Using a micropipette, add **248 µL** of siRNA Spark Formulation Buffer 2 to a RNase-free microcentrifuge tube.
3. Open the package containing a single siRNA Spark Cartridge and the accompanying Cartridge Cap inside the biosafety cabinet.
4. Using a micropipette, fill the largest well **(Well 1)** of the Cartridge with **124 µL** of Spark Formulation Buffer 2. To fill, place the pipette tip just above the hole at the bottom of the well and slowly dispense the solution into the well, avoiding bubbles during the process.
5. Fill the middle well **(Well 2)** of the Cartridge with **93 µL** of siRNA-Formulation Buffer 1 mix prepared in Section 5.1. To fill, place the pipette tip just above the hole at the bottom of the well and slowly dispense the solution into the well, avoiding bubbles during the process.
6. Fill the smallest well **(Well 3)** of the Cartridge with **31 µL** of Nanoparticle Mix. To fill, place the pipette tip just above the hole at the bottom of the well and slowly dispense the solution into the well, avoiding bubbles during the process.
7. Place the Cartridge Cap over the base of the Cartridge. Align the holes on the Cap with the posts on the Cartridge and insert into the instrument (follow instructions provided in the Spark User Guide).
8. Press the green-lit "Start" button on the base of the instrument.
9. Once the instrument completes its process, remove the Cartridge from the instrument and lift the Cap off carefully.
10. Using a micropipette, remove the entire contents from the largest well **(Well 1)** of the Cartridge and add it to the microcentrifuge tube containing 248 µL of the Formulation Buffer 2 prepared in Step 2 of Section 5.2. Label this as siRNA nanoparticles at a concentration of 130 µg/mL of siRNA.
    Note: The concentration of 130 µg/mL of siRNA is an estimation.
11. *Optional: To calculate the exact concentration and encapsulation efficiency of the siRNA nanoparticles, please refer to Section 6.1 - Appendix 1.*

Note: All siRNA nanoparticles manufactured on the Spark must be used within 14 days of manufacture.

Note: Should a larger volume of nanoparticles be required, separate encapsulations of the same nucleic acid sequence can be pooled together prior to treating cells. Pooling particles is recommended when using these nanoparticles in the same comparative experiment. Assume the encapsulated siRNA concentration of the pooled nanoparticles to be 130 µg/mL.

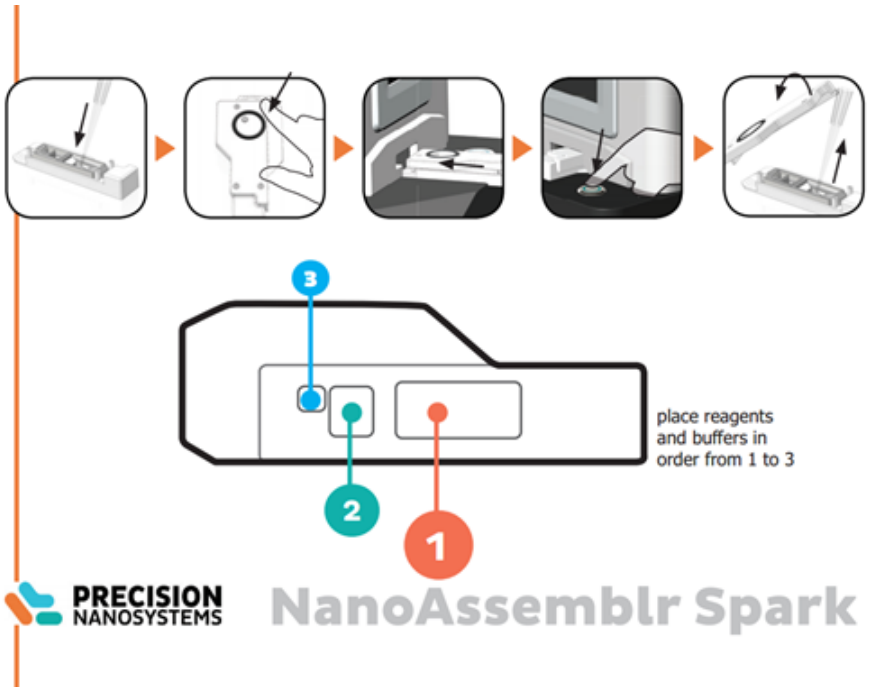


Figure 1. Illustrating the process for preparing siRNA nanoparticles using siRNA Spark Transfection Kits. Well 1 is filled with 124 µL of siRNA Spark Formulation Buffer 2, while well 2 is filled with 93 µL of the siRNA-Formulation Buffer 1 solution and well 1 is filled with 31 µL of the siRNA Spark Nanoparticle Mix. The filled cartridge is capped and placed in the Spark to manufacture the nanoparticles. These nanoparticles are in well 1 once the encapsulation is done.

6. Appendices

6.1. Appendix 1: Measure the Concentration of siRNA in the siRNA Nanoparticles using the Quant-iT™ RiboGreen® RNA Assay Kit

Measure the encapsulation efficiency and concentration of the siRNA in the siRNA-NPs using a suitable protocol such as the RiboGreen RNA Assay.
Note: PNI provides a protocol for quantifying siRNA concentration and encapsulation efficiency in siRNA nanoparticles using the Ribogreen RNA Assay. Please refer to the resource page on our website (https://www.precisionnanosystems.com/ resources/) to access the protocol for the Ribogreen Assay (PNI-SOP-NAS9-003-EXT).

Additional information about our products and instruments is available online. Please visit www.precisionnanosystems.com or call 1-888-618-0031 for technical assistance.

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