siRNA Spark™ Transfection Kit - 2nmol (2x)

This protocol provides instructions for the encapsulation of 2 nmol of siRNA into nanoparticles on the NanoAssemblr ™ Spark using various siRNA Spark Transfection Kits. Hepato9™, Neuro9™ and Test9™ 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.

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 2 The sterile pack contains a base referred to as the Cartridge and a cover termed the Cartridge Cap.	Sterile

Kits and Reagents	Special Instructions
Nucleic Acid Storage Buffer* – 200 µL	Sterile; Store at 4 °C
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 (Ex/Em: 644/665 nm) to visualize/quantify nanoparticle uptake in cells is included in the siRNA Spark Nanoparticle Mix. The emission spectrum is approximately 650 – 800 nm.
* Each vial has a descriptor (Neuro9, Hepato9, or Test9) 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)*
96-well Plate	Corning (cat # 3370)
siRNA	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)*

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

5. Protocol Instructions

Note: The protocol below outlines the steps required to encapsulate a 2 nmol sample of siRNA into nanoparticles. Please repeat the steps below to encapsulate the second sample of 2 nmol.
IMPORTANT: This protocol requires user to start with lyophilized siRNA.

5.1. Preparation of siRNA in Nucleic Acid Storage Buffer

Note: If the lyophilized siRNA amount is 2 nmol, please skip Section 5.1 and refer to Section 5.2. If starting amount of lyophilized siRNA is greater than 2 nmol, begin at Section 5.1
Note: Please maintain sterile conditions. Conduct all steps in a sterile biosafety cabinet.

1. Thaw and spin down the Nucleic Acid Storage Buffer and the lyophilized siRNA.
2. Add the required quantity of the Nucleic Acid Storage Buffer to the lyophilized siRNA to prepare an siRNA solution at a concentration of 20 mg/mL.
3. Pipette the solution up and down (>5 times) to ensure mixing of siRNA in the buffer.
   Note: siRNA can be stored in the Nucleic Acid Storage Buffer at -80 °C for subsequent use. The Nucleic Acid Storage Buffer cannot be used for manufacturing the siRNA nanoparticles.

5.2. Preparation of siRNA in Formulation Buffer 1

Note: Please maintain sterile conditions. Conduct all steps in a sterile biosafety cabinet.

1. Thaw and spin down the lyophilized siRNA, buffers and siRNA Spark Nanoparticle Mix vials. Pipet the Nanoparticle Mix up and down (>3 times) to mix.
2. If lyophilized siRNA amount is 2 nmol, add 36 µL of siRNA Spark Formulation Buffer 1 to the vial. Pipet the solution up and down (>5 times) to ensure mixing of siRNA in the buffer.
3. If siRNA concentration is at 20 mg/mL (from Section 5.1), add 1.8 µL to 37.2 µL of Formulation Buffer 1 in a RNAse-free microcentrifuge tube. Pipet the solution up and down >3 times to ensure mixing of siRNA in the buffer.
4. Measure the concentration of the siRNA-Formulation Buffer 1 solution with the NanoDrop™ 2000c UV-Vis spectrometer, using the Spark Formulation Buffer 1 as a blank. Acceptable range of siRNA concentration is 910 – 940 µg/mL.
5. If the concentration of siRNA is above the range, calculate and add the required amount of Formulation Buffer 1 to adjust the concentration to 930 µg/mL and repeat step 4 in Section 5.2.
   Note: Store the remaining Formulation Buffer 1 at 4 °C for the second encapsulation.

5.3. Manufacturing siRNA Nanoparticles

Note: A fluorescently-labeled dye (Ex/Em: 644/665 nm) to visualize/quantify nanoparticle uptake in cells is included in the siRNA Spark Nanoparticle Mix. The emission spectrum is approximately 650 – 800 nm.
Note: Please maintain sterile conditions. Conduct all steps in a sterile biosafety cabinet.

Note: Refer to Figure 1 for demonstration.

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 **96 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 **48 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.
   Note: Store the remaining Formulation Buffer 2 at 4°C for the second encapsulation.
5. Fill the middle well **(Well 2)** of the Cartridge with **36 µL** of siRNA-Formulation Buffer 1 prepared in Section 5.2. 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.
6. Fill the smallest well **(Well 3)** of the Cartridge with **12 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.
   Note: Store the remaining Nanoparticle Mix at -80 °C for the second encapsulation.
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 96 µL of the Formulation Buffer 2 prepared in Step 2 of Section 5.3. 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. This is especially important when using these nanoparticles in the same comparative experiment. Assume the encapsulated siRNA concentration of the pooled nanoparticles to be 130 µg/mL.

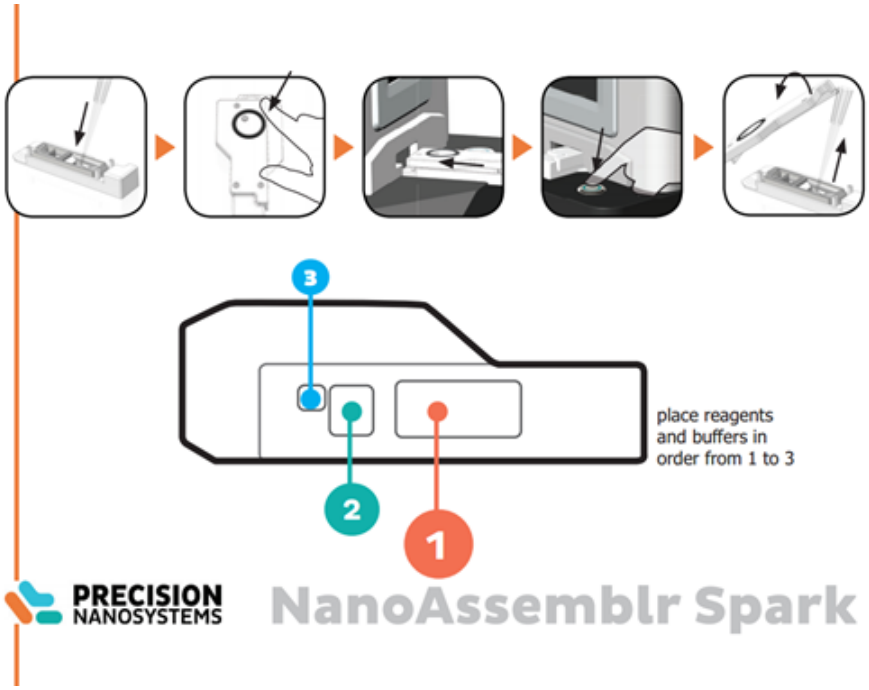

Figure 1. Illustrating the process for preparing siRNA nanoparticles using siRNA Spark Transfection Kits.
Well 1 is filled with 48 µL of siRNA Spark Formulation Buffer 2, while well 2 is filled with 36 µL of the siRNA-Formulation Buffer 1 solution and well 1 is filled with 12 µL of the siRNA Spark Nanoparticle 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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