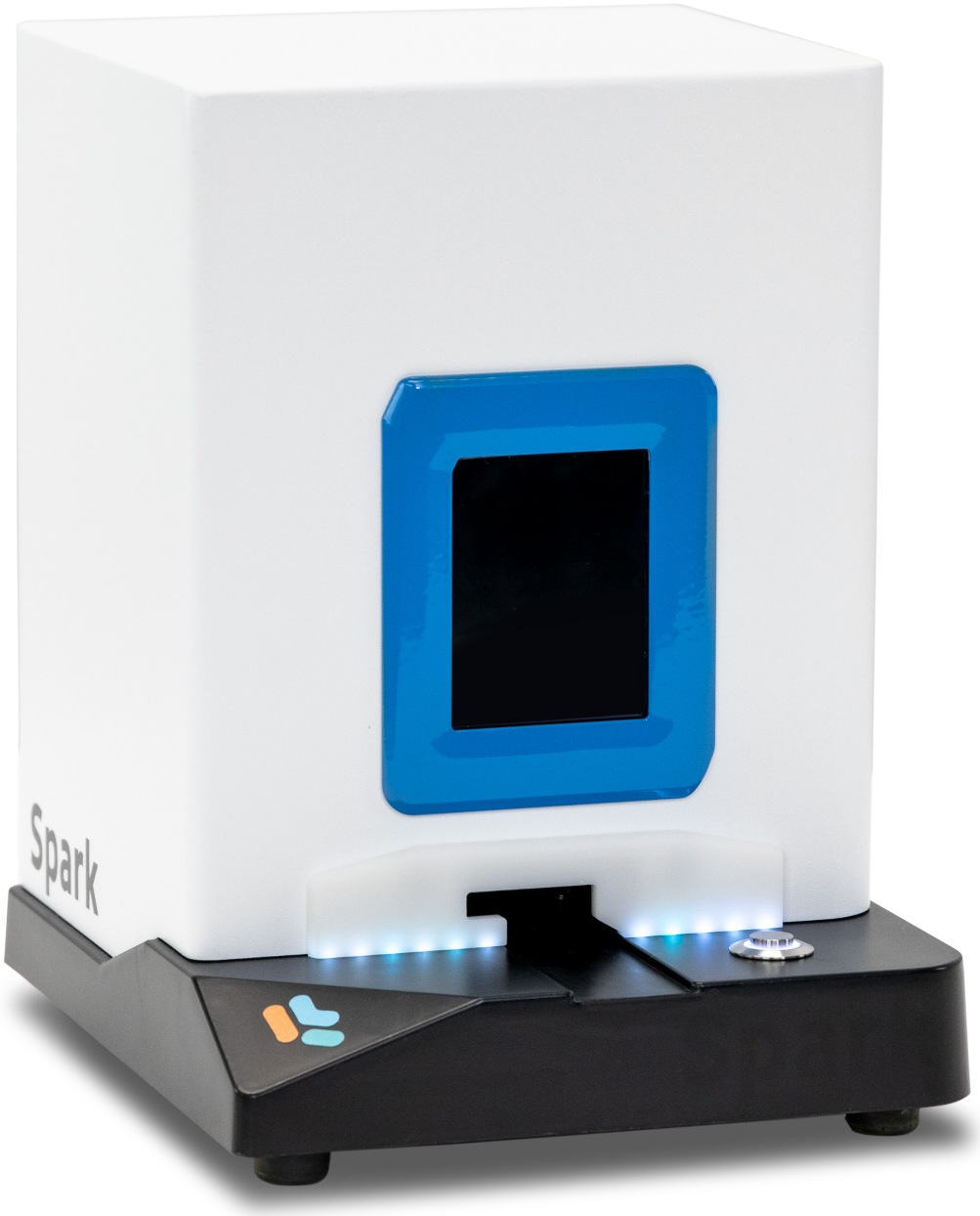
NanoAssemblr™ Spark™ User Guide

Power to Discover



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1 Introduction

NanoAssemblr™ Spark™ enables the acceleration of genetic medicine discovery and development through the rapid and easy ultra-low volume production of nanoparticles. Researchers are able to explore innovative nanomedicine formulations and create revolutionary genetic medicine with Spark's scalable and robust NxGen™ microfluidic system, with minimal consumption of starting materials. The Spark system is designed to be seamlessly integrated with *in vitro* screening workflows to create on-demand formulations in a sterile biosafety hood and apply them directly to cells in culture.

The Spark instrument streamlines the screening of various chemical formulation parameters for novel excipients and nanoparticle cargos. Precision NanoSystems' (PNI) line of Spark Kits can also be used to formulate particles for nucleic acid delivery on Spark.

2 Specifications

Electrical	100–240 VAC, 0.58 A (Max.)
Environmental Conditions	Temperature: 5–40 °C Humidity: 0–40% (non-condensing) Pressure: 70–106 kPa
Dimensions (Width x Depth x Height)	16.5 x 19.5 x 22.5 cm
Weight	3.6 kg
Cartridge	Spark NxGen single-use microfluidic cartridge
Formulation Volume	25–250 µL

Table 1: *Spark Specification*

3 Safety Considerations

3.1 Safe Operation

Ensure NanoAssemblr Spark is only used with cartridges, consumables and parts supplied by PNI directly or through an authorized distributor.

Use only power supplies and cords supplied by PNI with Spark. Contact PNI if a replacement is required.

3.2 Maintenance

NanoAssemblr Spark contains no user serviceable components. Preventative maintenance and service should only be performed by an authorized, trained representative of PNI. A yearly preventive service is recommended for Spark.

3.3 Symbols

WARNING! Risk of Danger.

Documentation should be consulted. Proceed with caution.

WARNING! Crush Hazard.

Keep hands clear from moving parts to avoid pinch injuries.

4 Components and Terminology

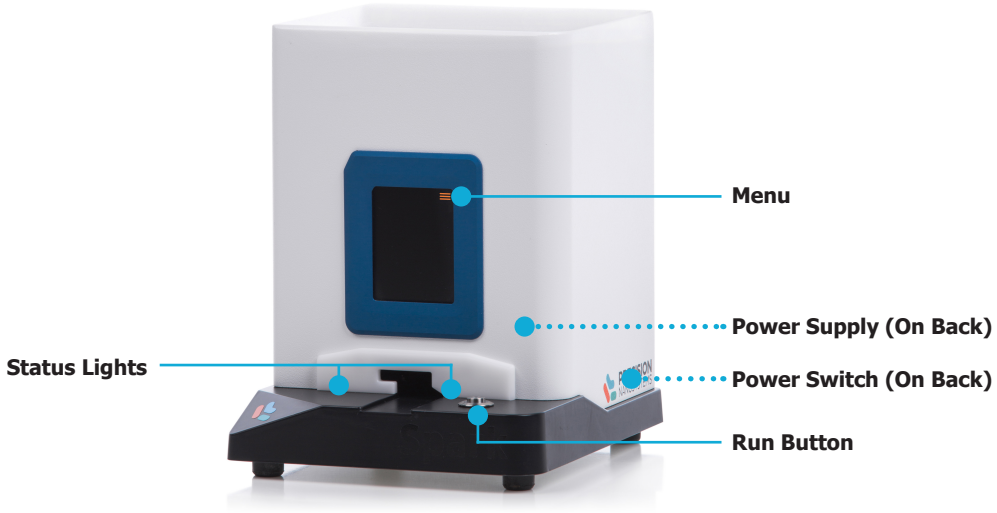


Figure 4-1: *NanoAssemblr Spark*

Figure 4-2: *The NanoAssemblr Spark Power Supply With Various Regional Plugs (Provided)*

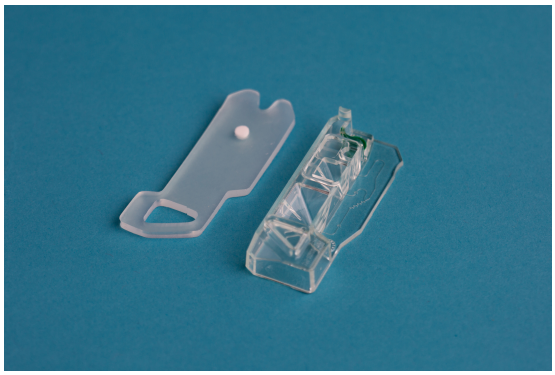


Figure 4-3: *The NanoAssemblr Spark Cartridge With Cap*

5 Installation

1. Unpack the NanoAssemblr Spark instrument and power supply.

   NOTE: Use only power cords supplied by PNI with Spark, contact PNI if a replacement is required.

2. Place Spark on a level surface with enough space around the instrument to allow for easy access to the Run Button, Power Switch and to disconnect the power supply should it be necessary.
   - Spark may be used inside a Biological Safety Cabinet (BSC), or similar setting with minimal disruption to the air flow pattern.
3. Connect the correct regional wall prong adapter to the power supply.
4. Connect the power supply to Spark.
5. Connect the power supply to a standard wall outlet.
6. Turn on the Power Switch.
7. The Status Lights will illuminate white when the instrument is ready for use.
8. If desired, the internal system can be purged with ambient air by tapping the *Menu* icon and selecting **Purge**. This is an optional step when installing Spark in a controlled environment such as a BSC or similar (see Section 6.2 Operating Modes for further information on Purging).

6 Operation

Spark is designed for use with Spark Cartridges. Spark Cartridges are provided independently or are included with some Spark kits.

6.1 Navigating the Spark Interface

Spark is equipped with an onboard touch interface, which can be navigated to select between formulation and purge mode via the *Menu* button in the top right corner of the main screen (**Figure 6-1**).

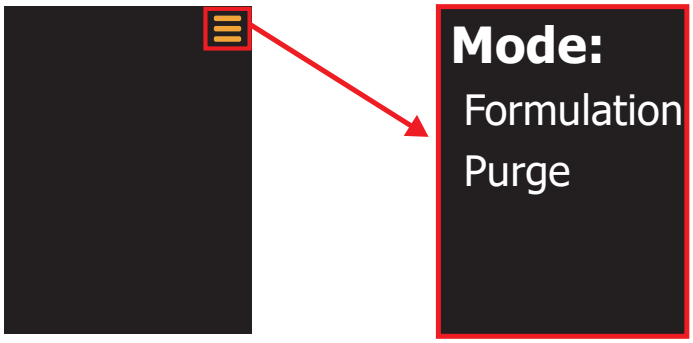


Figure 6-1: *The Menu Button Allows the Manual Selection of Operating Modes*

6.2 Operating Modes

There are two modes of operation on Spark (Table 2).

By selecting **Formulation Mode**, the user can select a *Setting* (1 to 10) for formulating nanoparticles with user-provided materials loaded into a Spark Cartridge. *Settings* correspond to the amount and duration of which the Spark Cartridge is pressurized by the instrument to facilitate the flow of fluids from the input wells to the output well. Refer to Section 6.3 Operating with a Spark Cartridge for more information.

When using the Neuro9™ siRNA Spark™ Kit or GenVoy-ILM™ T Cell Kit for mRNA, select **Formulation Mode** and consult the Spark Kit user guide for the recommended setting to use.

Figure 6-2: *Formulation Mode Requires the Selection of a Setting for Formulation*

Purge Mode is used to purge the internal system with ambient air (See Section 5 Installation). This may be desirable when the Spark is first installed in a sterile environment.

Figure 6-3: *Press on the Instrument Button to Purge Ambient Air*

Mode	Description
Formulation	Spark will be on standby for the insertion of a Spark Cartridge. This will allow the user to select the desired *Setting* to formulate nanoparticles with user-provided materials.
Purge	Spark will purge the internal system with ambient air.

Table 2: *The Operating Modes of Spark*

6.2.1 Formulation Mode

Input materials for lipid nanoparticle formulation on Spark include three solutions:

- An aqueous phase—nucleic acid solution in formulation buffer
- An organic phase—a lipid solution in organic solvent
- a dilution buffer

Spark operates using a pressure-driven mechanism, exerting the same pressure on both input wells of the Spark Cartridge. Physicochemical properties of the input materials, such as viscosity and temperature, will affect the specific *Setting* required to completely use all the material in the input wells. For a solution with an aqueous and organic phase, it is recommended to use 2:1 aqueous to organic phase volume ratio. Some adjustment of the aqueous to organic phase ratio may be required depending on the physicochemical properties of the solutions. For formulations with matching fluid phases (i.e., aqueous to aqueous), it is recommended to use a 1.6:1 volume ratio. To achieve optimal results, refer to Table 3 for suggested formulation volumes and corresponding *Settings* on Spark.

KEY CONSIDERATION

For a formulation on Spark, the N:P ratio (N = amines from cationic lipid and P = phosphates from nucleic acid) should be considered for determination of optimal charge interaction.

6.2.1.1 Optimizing Settings and Volumes for Nanoparticle Formulations

Optimization is required to determine the appropriate *Settings* and corresponding input volumes of aqueous and organic phases for preparing nanoparticles. The *Settings* (1 to 10) determine the duration and degree of Spark Cartridge pressurization, during which fluid from the input wells is pushed through the microfluidic mixing system to the output well. Physicochemical properties of the input materials, such as viscosity, will affect the specific *Setting* required to completely empty the input wells.

These *Settings* and input volumes may be modified to achieve optimal results with the input materials of choice. Some suggested input volumes of each component and their corresponding *Settings* are provided in Table 3. The recommended *Settings* are provided based on the final volume of nanoparticles required. To optimize the recovery of input materials, refer to the column on "Additional *Settings* to Test". Using the same volume of input materials with the full range of suggested *Settings* can help to identify the parameters required for optimal formulation and recovery.

It is recommended that optimization studies start at *Setting* "3" with 48 µL dilution buffer, 32 µL aqueous phase and 16 µL organic phase. With these input volumes, a further 1:1 dilution (optional, for *in vitro* applications) of the resulting nanoparticle solution is performed to achieve a final volume of 96 µL. To account for varying physicochemical properties (i.e., viscosity), the setting and input ratio may need to be optimized for the chosen aqueous and organic solutions (refer to Table 3).

Recommended Settings	Dilution buffer (µL)	Aqueous phase (µL)	Organic phase (µL)	Output volume (µL)	Final volume after optional 1:1 dilution (µL)	Additional *Settings* to test
1	24	16	8	48	96	1–3
3	48	32	16	96	192	2–4
5	72	48	24	144	288	4–6
7	96	64	32	192	384	6–8
9	120	80	40	240	480	8–10

Table 3: *Suggested Formulation Volumes Using the Spark Cartridge*

6.3 Operating Spark with a Spark Cartridge

To achieve the best results using Spark and to avoid premature flow into the microfluidic channels on the Spark Cartridge, it is important to use correct technique when pipetting fluids into a Spark Cartridge. When pipetting fluid into each well of the Spark Cartridge, the pipette tip should be positioned at the bottom of the wells between the wall and the microfluidic channel opening. A slow and steady pipetting technique should be used to avoid spilling and bubble formation. It is essential to check that no air is trapped beneath the dispensed fluid after pipetting into each well (**Figure 6-4**).

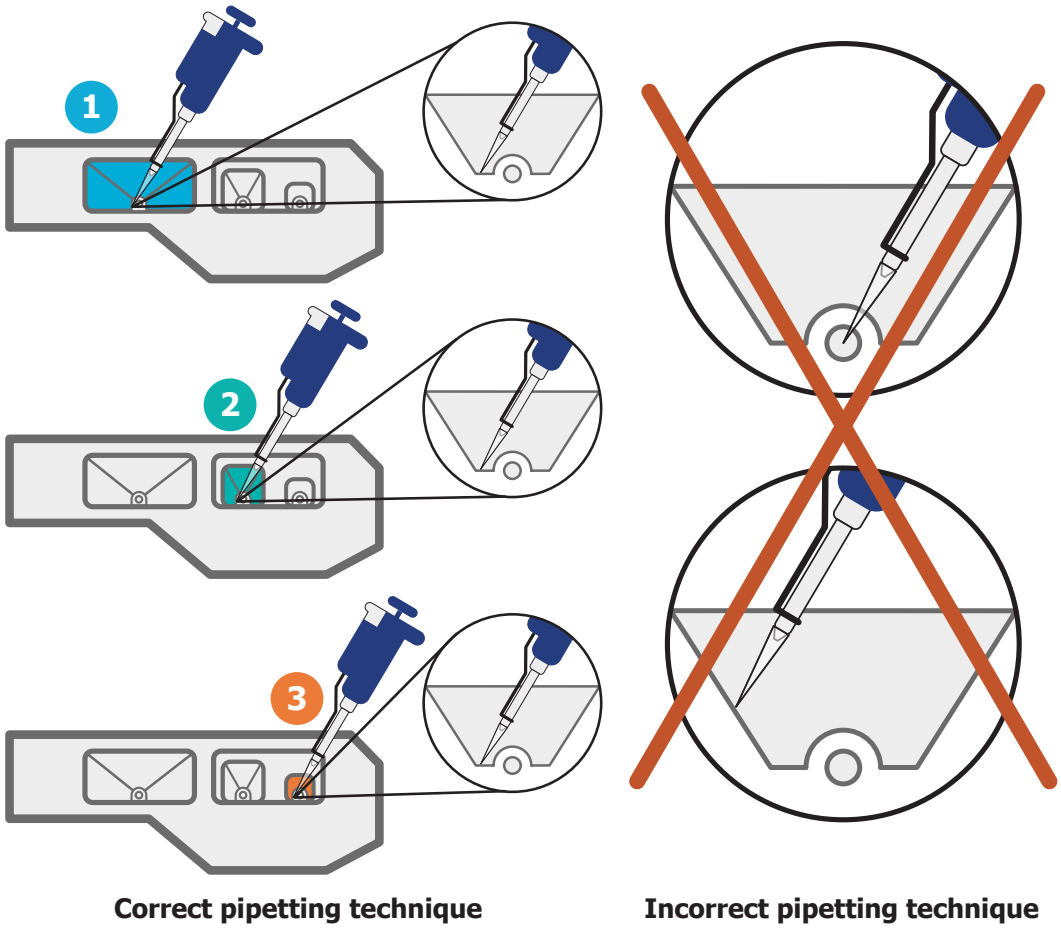


Figure 6-4: *Best Practices and Loading Order for Pipetting into a Cartridge*

Cartridges should be filled immediately before loading onto the Spark instrument; prefilling of cartridges in advance (e.g., for performing multiple formulations) is not recommended.

To perform a formulation on Spark, load fluids into the wells in descending order of volume (Wells 1, 2 and 3 consecutively):

1. Dispense the required Dilution Buffer into the largest well (Well 1) (**Figure 6-4**).
2. Dispense the aqueous phase containing the nucleic acid in the appropriate formulation buffer into the middle well (Well 2).

3. Dispense the organic phase containing lipids into the smallest well (Well 3).
4. After pipetting all components, check for any bubbles present at the bottom of the inlet wells. Use a sterile needle to remove air bubbles from the inlet wells before inserting the cartridge into the instrument.
5. Place the cap securely over the cartridge.

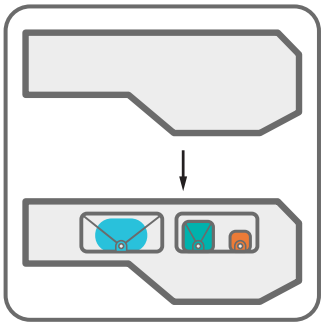


Figure 6-5: *Capping the Spark Cartridge*

6. Insert the capped cartridge into Spark (**Figure 6-6**). The Status Lights will illuminate green when the cartridge is successfully inserted and detected by the instrument.

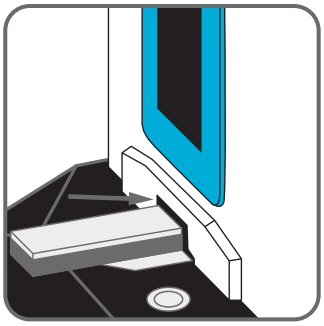


Figure 6-6: *Inserting the Cartridge into Spark*

7. From the *Menu*, select **Formulation** mode. Then, set the appropriate Setting using the **+** and **–** buttons on the Spark screen (**Figure 6-7**). Suggested input volumes and their corresponding recommended *Settings* are shown in Table 3.

Figure 6-7: *User Selects a Setting (1 to 10) to Use in Formulation Mode*

8. Press the Run Button once.
9. The start of the formulation process will be indicated on the screen.

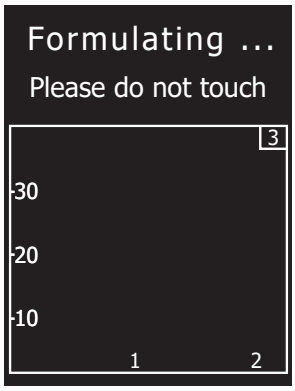


Figure 6-8: *The Initial Screen Will Indicate Formulation and Display an Empty Graph*

10. A live graph of the AIR pressure throughout the formulation will be shown on the screen. (See Section 7 Live Pressure Graph)
11. The screen will then indicate the completion of the formulation. Graphs will vary, please refer to Section 7: Live Pressure Graph for more information.

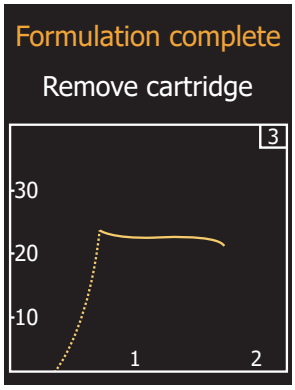


Figure 6-9: *The Spark Screen Upon Completion of a Formulation*

12. Remove the cartridge from the instrument and take off the lid from the cartridge to access your formulation.
13. Collect the formulation from the outlet well using a pipette (**Figure 6-10**).

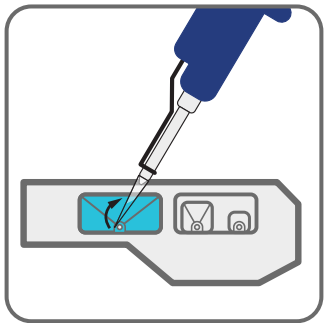


Figure 6-10: *Collecting the Formulated Particles Using a Pipette*

14. *Optional:* Perform a further 1:1 dilution of the nanoparticle formulation with Dilution Buffer in a separate sterile collection tube.

    For example, for a formulation using 16 µL Lipid Solution, 32 µL Formulation Buffer and 48 µL Dilution Buffer, the volume collected in the outlet well will be approximately 96 µL. Pipette this volume from the outlet well and add to a labelled collection tube containing 96 µL of Dilution Buffer.

15. The nanoparticle formulation is now ready for downstream assays and other applications.

7 Live Pressure Graph

7.1 Formulation Feedback

Spark operates using an air pressure-driven mechanism. During the formulation process, the pressure is monitored by the instrument's internal sensors and displayed on the screen. The y-axis represents the pressure as measured in PSI and the x-axis represents time as measured in seconds. The number in the box at the top right hand corner of the graph denotes the setting used for the formulation.

While the graphical representation of the pressure throughout each formulation can vary, in general, the pressure should increase quickly to a peak and then decrease slightly to a plateau until the formulation is complete as displayed in the image below.

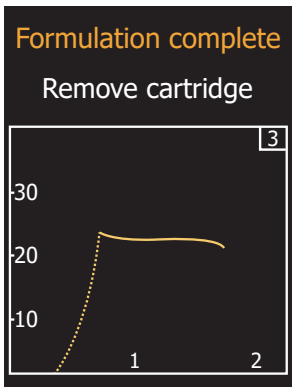


Figure 7-1: *The End Screen Will Indicate the End of Formulation and Display a Full Graph*

7.2 Pressure Test

If you would like to test whether the air pump for your Spark instrument is performing properly, you can conduct a pressure test using a specialized Pressure Test Cartridge at setting 10. A Pressure Test Cartridge can be purchased with the instrument service plan and can be used 100 times before a new Pressure Test Cartridge should be ordered from PNI. On a correctly functioning Spark instrument at setting 10, the live pressure graph during a pressure test displays a peak above 25 PSI that quickly achieves steady state between 25-31 PSI (**Figure 7-2**). The yellow number displayed on the graph when using a Spark instrument at setting 10 can be communicated to the PNI Service and Maintenance team to help diagnose any problems.

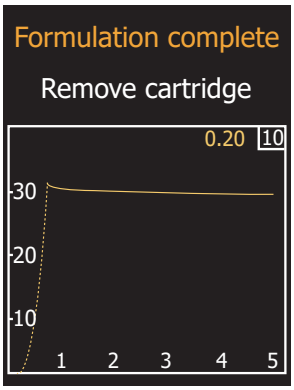


Figure 7-2: *The Final Screen When Performing a Pressure Test*

Pressure tests must be done at setting 10, otherwise the live pressure graph will appear differently than above (**Figure 7-3**).

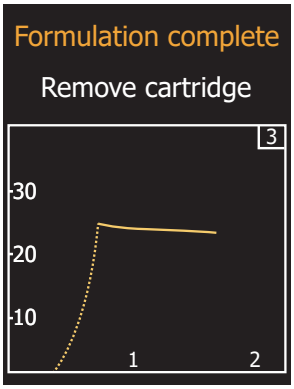


Figure 7-3: *Using a Specialized Pressure Test Cartridge at Setting 3 Will Not Provide a Useful Live Pressure Graph*

8 Troubleshooting

8.1 Interface Observations

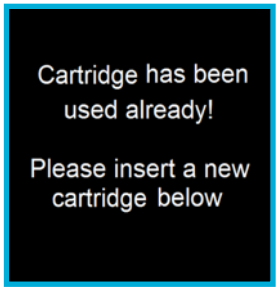
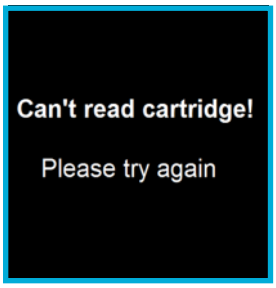
Observation	Potential Cause & Recommended Solution
 	🔴 Light Status: Red • The cartridge is used or invalid. Spark Cartridges are single use. Please use a new Cartridge for each formulation. • The cartridge is not properly inserted. Please remove the cartridge, reinsert it and ensure the Status Light illuminates green prior to formulation.
	🟡 Light Status: Yellow • There was an error reading the cartridge. Please fully remove the cartridge and re-insert it. • Spark was powered on with a cartridge inserted. Fully remove the cartridge and power the instrument off. Turn the instrument back on and re-insert a cartridge when prompted.

Table 4: *Troubleshooting Errors Indicated by the Spark Interface*

8.2 Cartridge Observations

Observation	Potential Cause & Recommended Solution
Left over volume in both wells	**Reason:** *Setting* is too low. **Explanation:** The pressure in the wells and the pressurization times are too low to empty the input wells. **Solution:** Increase the *Setting* value while maintaining the same input volumes.
Left over volume in one well	**Reason 1:** Premature flow from one of the wells during the pipetting step. **Explanation:** If one well empties before the other, air will enter the channels and inhibit flow from the well that still contains fluid. **Solution:** When dispensing the fluid into the well, keep the pipette tip on the bottom surface close to the wall of the input well (See Section 6.3 Operating Spark with a Spark Cartridge). Ensure no fluid enters the microfluidic channels.
	Reason 2: Volume ratio does not match. **Explanation:** Properties of the input materials such as viscosity can affect the dynamics of the fluid path through the cartridge channels. **Solution:** Change the starting volume ratio by either increasing the input volume to the well that had no residual volume or decreasing the input volume to the well that contained leftover volume.
White residue	**Reason:** Premature flow from one of the wells during the pipetting step. **Explanation:** This premature flow results in early non-controlled mixing of the aqueous and organic phase, causing the lipids to precipitate out of solution and resulting in the presence of a white residue. **Solution:** When dispensing the fluid into the well, keep the pipette tip on the bottom surface close to the wall of the input well (See Section 6.3 Operating Spark with a Spark Cartridge). Ensure no fluid enters the microfluidic channels.

Table continues on next page...

Bubbling, spills	**Reason 1:** Premature flow from one of the wells during the pipetting step. **Explanation:** If one well empties before the other, air will enter the channels and bubbles will appear in the output well. **Solution:** When dispensing the fluid into the well, keep the pipette tip on the bottom surface close to the wall of the input well (See Section 6.3 Operating Spark with a Spark Cartridge). Ensure no fluid enters the microfluidic channels.
	Reason 2: *Setting* is too high. **Explanation:** The pressure in the wells and the pressurization times are too high, resulting in emptying of the wells and air flow into the output well and bubbling/spilling of the formulation. **Solution:** Decrease the Setting value while maintaining the same input volumes.

Table 5: *Troubleshooting Errors with the Spark Cartridge*

Please contact one of our Field Application Scientists for further assistance on nanoparticle formulation applications with NanoAssemblr Spark.

8.3 Live Pressure Graph Observations

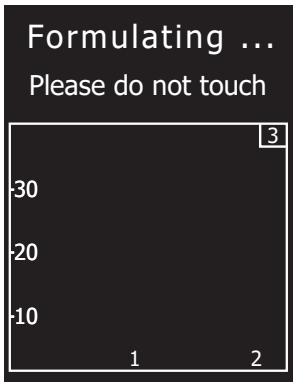
Observation	Potential Cause & Solution
	A blank graph indicates a misplaced, damaged or forgotten cartridge lid. Please double check the cartridge lid and try again.

Table continues on next page...

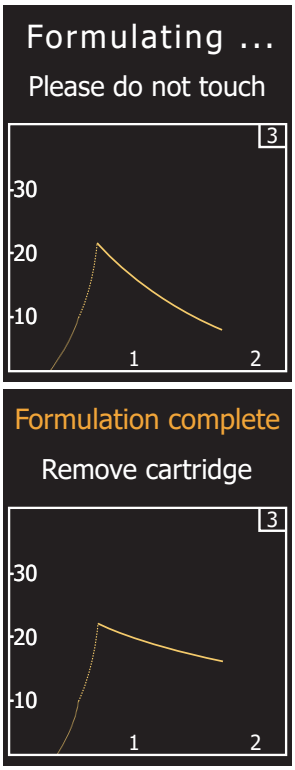
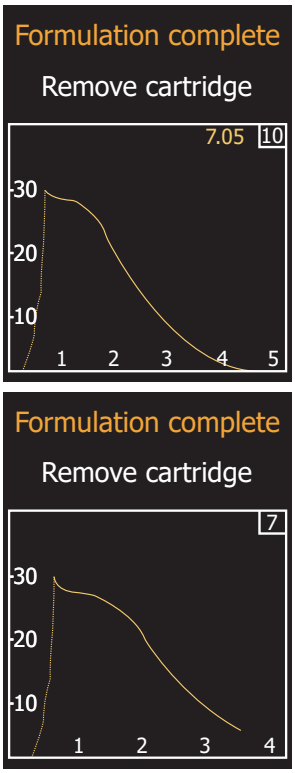
	A peak and then a quickly decreasing pressure line indicates a lack of liquid in one or more of the wells. First image is no liquid in any well, second image is liquid in one well (with dilution). Please increase the fluid added to each input well or use a lower setting.
	A line that peaks, reaches a plateau but decreases before the end of the graph indicates that air is being pushed through the wells before the end of the formulation process. This happens when too little fluid is input, or using a setting that is too high. This is commonly seen alongside splashing or bubbles. First image is at setting 10, second image is at setting 7. Please increase the fluid added to each input well or use a lower setting.

Table 6: *Troubleshooting Using the Live Pressure Graph*

9 Cleaning, Decontamination and Disposal

It may be desirable or necessary to periodically clean the outside of Spark instrument (e.g., when moving the instrument into a sterile environment). In such a case, the outside of the instrument may be wiped down with 70% ethanol.

WARNING! Do not spray the instrument directly. Spray 70% ethanol onto a cloth or tissue to wipe down the instrument. Do not apply excessive force on the screen when wiping down the instrument.

NanoAssemblr Spark users should be aware of the materials used in formulations and their specific risks and hazards. Prior to any service by PNI, a completed Decontamination Form must be submitted to PNI Technical Support. This form will be provided when service arrangements are made. Spark may be decontaminated using a mild, non-abrasive soap or up to 70% ethanol. For further information on the compatibility of a particular decontamination agent, please contact PNI Technical Support.

Should it be necessary to dispose of Spark, local e-waste regulations must be followed. Users are encouraged to consult their company or institution and follow implemented procedures for disposal of lab equipment.

10 Regulatory

10.1 Brazil

Incorpora produto homologado pela Anatel sob número 21093-22-12931.

Este equipamento não tem direito à proteção contra interferência prejudicial e não pode causar interferência em sistemas devidamente autorizados.

Para mais informações, consulte o site da Anatel: https://www.gov.br/anatel/pt-br
Anatel
Evento discutirá aspectos jurídicos e regulatórios da regulação consumerista de telecomunicações

10.2 Thailand

เครื่องโทรคมนาคมและอุปกรณ์นี้มีความสอดคล้องตามข้อกำหนดของกสทช.
This telecommunication equipment is in compliance with NBTC requirements.

This radio communication equipment is exempted to possess a license, user license, or radio communication station license as per NBTC notification regarding radio communication equipment and radio communication station has been exempted for license according to radio communication act B.E.2498.

10.3 Singapore

Complies with
IMDA Standards
DA103787

11 Ordering Information

Image	Name	Product Code	Includes
	NanoAssemblr™ Spark™ Instrument	NIS0001	1 NanoAssemblr™ Spark™ Instrument 1 Power supply (worldwide) 1 One-year warranty
	NanoAssemblr™ Spark™ Cartridges* - 20 pack NanoAssemblr™ Spark™ Cartridges* - 80 pack	NIS0009 NIS0013	20 Cartridges 80 Cartridges
	NanoAssemblr™ Spark™ Demo Kit - mRNA	NCS0017	5 Spark Cartridges Nanoparticle Mix mRNA formulation buffer 1 mRNA formulation buffer 2
	NanoAssemblr™ Spark™ Pressure Test Cartridge	1003010	1 specialized Spark™ cartridge

Table 7: *Ordering information: Instrument and Consumables (*Single-Use Cartridges)*

Service:

Name	Product Code	Includes
NanoAssemblr Spark Service - Total Service Plan	NOB0004	Pre-paid service product and priced per year. The Total Service Plan includes prioritized technical assistance via telephone and/or on-site, repair of instrument defect, including spare parts, one preventative maintenance service per year.
NanoAssemblr Spark Service - Preventative Maintenance	NOS0006	One-time pre-paid service product. Preventative Maintenance provides a general service on the instrument.

Table 8: *Ordering Information: Services*

For NanoAssemblr™ Spark™ Reagents and Kits refer to precisionnanosystems.com/platform-technologies/product-comparison/spark#accessories.

To learn more about NanoAssemblr Spark, see a demo video, or reach a specialist, visit precisionnanosystems.com/platform-technologies/product-comparison/spark.

Or to learn more about the NanoAssemblr Platform, visit precisionnanosystems.com/platform-technologies/product-comparison.

Failure to follow the steps and guidelines listed in this user guide can result in instrument malfunction, suboptimal performance, property damage, and even injury to the user.

About Precision NanoSystems

Precision NanoSystems is a global leader in ushering in the next wave of genomic medicines in infectious diseases, cancer and rare diseases. We work with the world's leading drug researchers to understand disease and create the therapeutics and vaccines that will define the future of medicine. Precision NanoSystems offers proprietary technology platforms and comprehensive expertise to enable researchers to translate disease biology insights into non-viral genomic medicines.

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Product Number: NIS1024