GenVoy-ILM™
User Guide

For use with the
NanoAssemblr® Ignite™ & the
NanoAssemblr® Benchtop™

NIN1182

Contents

Description

GenVoy Ionizable Lipid Mix (ILM) is the simplest way to make lipid nanoparticles (LNPs), which are the most advanced technology for non-viral nucleic acid delivery. The key innovation is a cationic ionizable lipid. This class of lipid has been shown in literature to be important for:

- High nucleic acid encapsulation efficiency
- High transfection efficiency
- Low toxicity in primary cells and *in vivo*

Genvoy LNPs feature a homogeneous core structure and are formed when mixing GenVoy-ILM™ and nucleic acids using NanoAssemblr® microfluidic mixing technology under time invariant conditions. Visit the following page for more details:

www.precisionnanosystems.com/areas-of-interest/formulations/lipid-nanoparticles

LNPs mimic low density lipoproteins (LDL) and are taken up by receptor-mediated endocytosis. Once in the cell, ionizable lipids sense the pH change and trigger the disruption of the endosome to release the nucleic acid into the cytoplasm. To enter the endocytic pathway, LNPs interact with apolipoproteins which are the substrate for the LDL-receptor. Apolipoproteins are naturally available in systemic circulation *in vivo*, but may or may not be present in cell culture. The LDL receptor-mediated pathway is present in many cell types.

Product Components

Component	mol%	
	GenVoy-ILM	GenVoy-ILM with Dye
PNI Ionizable Lipid	50	50
DSPC	10	10
Cholesterol	37.5	37.4
PNI Stabilizer	2.5	2.5
DiD (Fluorescent Dye)	0	0.1

Other Details

Product	GenVoy-ILM	GenVoy-ILM with Dye
Avg MW (g/mol)	630.5	631
Concentration (mM)	25	25

PNI Formulation Buffer

Concentration (mM)	100
pH	4.0

Storage Conditions

Product	Long Term Storage* (>3 months)
GenVoy-ILM™	-80 °C
GenVoy-ILM™ with dye	-80 °C, Protect from light
PNI Formulation Buffer	-80 to 4 °C

**For short term storage (<3 months), store at -20°C or below. Shipping conditions may differ from storage conditions listed above, but will not affect product performance.*

Optical Properties

Product	Excitation Wavelengh (nm)	Emission Wavelength (nm)
GenVoy-ILM™ with Dye	644	665

General Product Use Limitations and Warranty

GenVoy-ILM™ is intended for research use only. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease. GenVoy-ILM™ is subject to PNI's general terms and conditions which can be found at:

www.precisionnanosystems.com/terms-and-conditions/

GenVoy-ILM - Expected Performance

Particle characteristics can vary depending on formulations conditions, N/P ratio, RNA size, etc. When using PNI Formulation Buffer, and following the provided protocol, with the specified RNA, the expected range of typical characteristics as measured by dynamic light scattering are tabulated below.

Payload	Z-Avg Diameter	PDI
Factor-VII siRNA	45–75 nm	< 0.15
Luciferase mRNA (~2000 nt)	60–120 nm	< 0.25

General Considerations

RNA-LNPs are prepared by mixing the ethanolic solution of lipids with an aqueous solution of RNA at low pH. Upon mixing, the change in polarity of the environment triggers the self-assembly of LNPs. The low pH causes the ionizable lipids to become cationic, where they first interact with anionic RNA through electrostatic complexation to form the particle core. Other lipids assemble around the core.

Rapid mixing ensures homogeneous conditions and promotes core formation over growth, leading to a homogenous population of LNPs.

An important factor in determining the encapsulation efficiency and biological activity of LNP formulations is the N/P ratio. This is the molar ratio between amines (N, which become cationic at low pH) found on the ionizable lipids, and the phosphates (P, anionic) found on the RNA backbone.

Optimal N/P ratios need to be determined for maximizing RNA encapsulation and gene delivery. As a general guideline, higher N/P ratios are recommended for mRNA compared to siRNA. Other parameters that need to be optimized for biological activity in the intended cellular target include the size and sequence of the nucleic acid payload.

Among other factors, mixing parameters may influence characteristics such as particle size and PDI. The total flow rate (TFR) and the Flow Rate Ratio (FRR) are the most influential mixing parameters to tune for achieving optimal particles for a given application. In general, higher TFR and FRR result in smaller particle sizes.

The relationship between the working concentrations of the nucleic acid and the ionizable lipid mix in this formulation is described by this equation:

$$[n] = \frac{[L] \cdot X}{FRR \cdot \frac{N}{P}}$$

Where [n] is the concentration of mRNA/siRNA, [L] is the lipid mix concentration, and X is the mole fraction of ionizable cationic lipid. The above equation shows that FRR, N/P ratio, [L] and [n] are all related to one another. Higher FRR and N/P ratios call for lower concentrations of RNA. It is critical to consider this relationship when formulating nanoparticles when using GenVoy-ILM.

LNP formulation on the NanoAssemblr® Ignite™

Required PNI Products

Product Name	PNI Catalog Number
GenVoy-ILM™ (2mL or 5mL)	NWW0041 or NWW0042
GenVoy-ILM™ with Dye (2mL or 5mL)	NWW0039 or NWW0040
PNI Formulation Buffer	NWW0043
NanoAssemblr® Ignite™ NxGen™ Cartridge	NIN0061 (100 pack) or NIN0062 (200 pack)
NanoAssemblr® Ignite™ Instrument	NIN0001

Suggested Supplies and Equipment

To prepare RNA-LNPs with GenVoy-ILM the following third-party supplies and equipment are required:

Description	Recommended Product/Supplier
1mL Disposable Syringe*	Becton Dickinson Plastipak™ Luer Slip Tip
3mL Disposable Syringe*	Becton Dickinson Plastipak™ Luer-Lok™ Tip
Blunt Needles 18 Gauge	Becton Dickinson
Syringe filters - 0.2µm	Acrodisc®, 13mm, Supor® membrane
Centrifugal filter concentrator – 10kDa MWCO	Millipore®Amicon® Ultra-15
UV Spectometer	Thermo Fisher Nanodrop®
Bench Top Centrifuge	Major laboratory supplier (MLS)
RNAse free conical collection tubes 15 mL	15 mL Centrifuge Tube
RNAse free conical collection tubes 50 mL	50 mL Centrifuge Tube

**Please see Appendix B for expanded syringe compatibility on the NanoAssemblr® Ignite™ instrument*

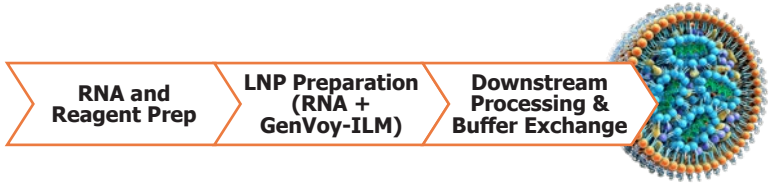
Suggested Reagents

Description	Recommended Product/Supplier
siRNA	Integrated DNA Technologies – Dicer Substrate siRNA
mRNA	TriLink CleanCap® mRNA
RNAse-free Phosphate Buffered Saline	Corning Dulbecco's PBS, 1x without calcium and magnesium
Apolipoprotein-E4	PeproTech Recombinant Human ApoE4
Absolute Ethanol	Major laboratory supplier (MLS)

Example Protocol for Formulating RNA Lipid Nanoparticles

The Ignite can prepare formulation volumes of 1–20 mL. This is an example of how to prepare a 2 mL RNA-LNP formulation using GenVoy-ILM on the Ignite.

Process overview:



Genvoy LNP

RNA and Reagent Prep

The following are guidelines to prepare working solutions of RNA in PNI Formulation buffer and how to prepare working solutions of GenVoy-ILM.

1. Clean the working area thoroughly with 70% ethanol.
2. Prepare GenVoy-ILM working solution. The recommended working concentration for GenVoy-ILM is 12.5 mM which can be achieved by diluting one-to-one with anhydrous ethanol. GenVoy-ILM can also be used up to the stock concentration.
3. Prepare the RNA working solution in PNI Formulation Buffer. RNA concentration will depend on GenVoy-ILM concentrations, the Flow Rate Ratio and the N/P ratio. The following is an initial guideline for GenVoy-ILM concentration of 12.5 mM, FRR of 3:1 and N/P ratio of 3 or 4 for siRNA and mRNA respectively.

Formulation type:	siRNA-LNP	mRNA-LNP
Working RNA Concentration Range (mg/mL)*	0.220–0.240	0.170–0.180
Working Buffer Concentration (mM)	25	Minimum 65

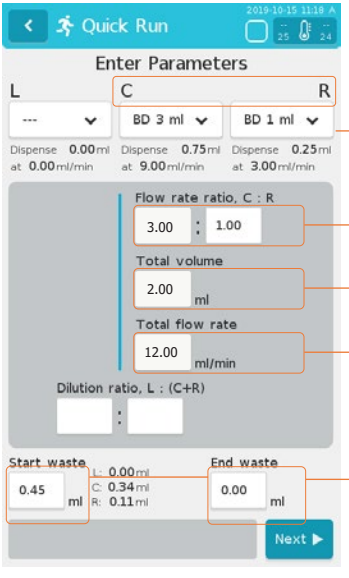
**Refer to Appendix A for tabulated RNA concentrations.*

4. Confirm RNA concentrations using a NanoDrop® UV spectrometer, with PNI Formulation Buffer as the reference blank.

RNA-LNP Preparation

Note: *Refer to the Ignite User Guide for detailed operating instructions.*

1. Turn on the NanoAssemblr Ignite and select "Quick Run" from the Main Menu. Enter the parameters as shown below by selecting a field, selecting a value from the drop down menu OR entering the number with the onscreen keyboard and then tapping the check mark. Repeat with the next field until the screen looks the same as below:



1. Select syringe brand and then size as shown
2. Enter Flow Rate Ratio as shown
3. Enter Total Volume as shown
4. Enter Total Flow Rate as shown
5. Select syringe brand and then size as shown

2. Open the lid of the Ignite and ensure the Cartridge Adaptor is installed over the "L" inlet of the cartridge slot with the arrow facing upwards, then remove a NxGen™ cartridge from the package and insert it in the cartridge slot. Raise the rotating block until the cartridge luers are visible.
3. Draw at least 1.5 mL prepared RNA working solution into a 3 mL syringe. Use a blunt needle if necessary. Remove the needle, clear air bubbles from the syringe and use the plunger to advance the liquid to the tip, avoiding drips from the syringe tip. Insert the syringe into the "C" inlet of the Ignite Cartridge and twist clockwise to engage the Luer Lock.
4. Draw at least 0.5 mL prepared GenVoy-ILM solution into a 1 mL syringe using a blunt needle if necessary. Remove the needle, clear air bubbles from the syringe and use the plunger to advance the liquid to the tip, but avoid drips from the syringe tip. Insert the syringe into the "R" inlet of the Ignite Cartridge.
5. Return the rotating block to the downwards position. Ensure the sample switch arm for two 15 mL conical tubes is installed.
6. Mark a 15 mL conical collection tube as "RNA-LNP" and push the tube into the clip labeled "Sample". Mark another with "waste", and push the tube into the clip labeled "Waste".

7. Close the Ignite lid and tap "Next" on the screen. Confirm the parameters, read and confirm the information in the dialog box and press "Start". The pushers of the Ignite™ are now injecting the fluids into the microfluidic cartridge. The formulation is collected in the tube labeled "RNA-LNP".
8. After the motors have positioned themselves back in the home position, the screen will indicate when it is safe to open the lid. Open the lid and remove the conical collection tube labeled "RNA-LNP" and set aside for characterization and further processing immediately.
9. Raise the rotating block and remove the syringes from Ignite™ and discard them. Return the rotating block to the downward position and remove and discard the NxGen cartridge.
10. To make additional samples, tap the back " < " button to return to the Quick Run Screen and repeat Steps 3–10.
11. Measure the size of the particles and their polydispersity index (PDI) by DLS, use an aliquot of the sample and dilute 1:10 in PBS as per the PNI Sizing Protocol.
12. Dilute the prepared particles 40x in sterile Ca^{2+}- and Mg^{2+}-free PBS.
13. Fill the top compartment of an Amicon® Ultra-15 centrifugal filtration tube with the diluted particle solution. Spin the tube at 2000 x g for 30 minutes at 20°C.
14. Remove the solution below the filter unit and repeat as needed until the solution is re-concentrated to the original volume (2 mL).

Note: *To maximize recovery, rinse the centrifugal filtration membrane by gently pipetting the retained sample solution over the membrane several times to dislodge any particles adsorbed to the membrane before recovering or adding additional sample.*

15. Use an Acrodisc® filter (0.2 µm pore size) to sterile-filter the concentrated RNA-LNP sample. Some formulations with larger particles or poor PDI may require prefiltering with 0.45 µm pore size.

Note: *Other sterile filters may interact with RNA-LNPs.*

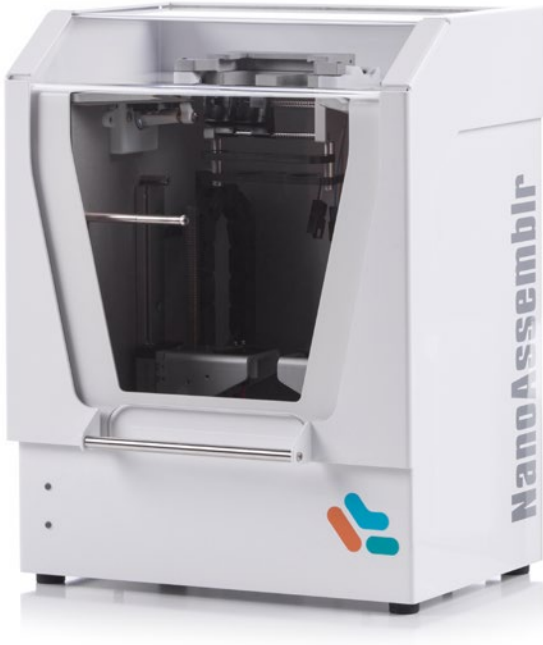
16. Remeasure the size of your particles and the PDI by DLS as performed in Step 11.
17. Measure the mRNA concentration using the RiboGreen® Assay as per the PNI RiboGreen® protocol (Appendix C). A PNI representative will provide the RNA Quantification Workbook spreadsheet to calculate the RNA concentration.

Related Documents

- NanoAssemblr® Ignite™ User Guide (Ignite-UG-1019)
- RNA Quantification workbook (PNI-WB-S9-001-INT)
- PNI Sizing protocol (PNI-SOP-UN-002-EXT)

Note: *If you did not receive these related documents contact your Field Application Scientist or email us at info@precision-nano.com*

LNP formulation on the NanoAssemblr® Benchtop™



Required PNI Products

Product Name	PNI Catalog Number
GenVoy-ILM™ (2mL or 5mL)	NWW0041 or NWW0042
GenVoy-ILM™ with Dye (2mL or 5mL)	NWW0039 or NWW0040
PNI Formulation Buffer	NWW0043
NanoAssemblr® Benchtop™ Microfluidic Cartridge	NIT0062 (100 pack)
NanoAssemblr® Benchtop™ Instrument	NIT0013

Suggested Supplies and Equipment

To prepare RNA-LNPs with GenVoy-ILM the following third-party supplies and equipment are required:

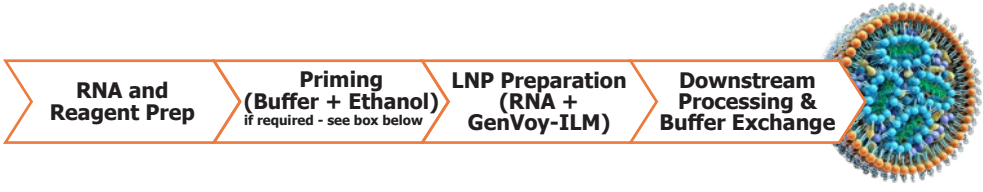
Description	Recommended Product/Supplier
1mL Disposable Syringe	Becton Dickinson Plastipak™ Luer Slip Tip
3mL Disposable Syringe	Becton Dickinson Plastipak™ Luer Slip Tip
Blunt Needles 18 Gauge	Becton Dickinson
Syringe filters - 0.2µm	Acrodisc®, 13mm, Supor® membrane
Centrifugal filter concentrator – 10kDa MWCO	Millipore®Amicon® Ultra-15
UV Spectometer	Thermo Fisher Nanodrop®
Bench Top Centrifuge	Major laboratory supplier (MLS)
RNAse free conical collection tubes 15 mL	15 mL Centrifuge Tube
RNAse free conical collection tubes 50 mL	50 mL Centrifuge Tube

Suggested Reagents

Description	Recommended Product/Supplier
siRNA	Integrated DNA Technologies – Dicer Substrate si-RNA
mRNA	TriLink CleanCap® mRNA
RNAse-free Phosphate Buffered Saline	Corning Dulbecco's PBS, 1x without calcium and magnesium
Apolipoprotein-E4	PeproTech Recombinant Human ApoE4
Absolute Ethanol	Major laboratory supplier (MLS)

Example Protocol for Formulating RNA Lipid Nanoparticles

The Benchtop can make formulation volumes of 1–15 mL. This is an example of how to prepare a 2 mL RNA-LNP formulation using GenVoy-ILM on the Benchtop.



Genvoy LNP

RNA and Reagent Prep

The following are guidelines to prepare working solutions of RNA in PNI Formulation buffer and how to prepare working solutions of GenVoy-ILM.

1. Clean the working area thoroughly with 70% ethanol.
2. Prepare GenVoy-ILM working solution. The recommended working concentration for GenVoy-ILM is 12.5 mM which can be achieved by diluting one-to-one with anhydrous ethanol. GenVoy-ILM can also be used up to the stock concentration.
3. Prepare the RNA working solution in PNI Formulation Buffer. RNA concentration will depend on GenVoy-ILM concentrations, the Flow Rate Ratio and the N/P ratio. The following is an initial guideline for GenVoy-ILM concentration of 12.5 mM, FRR of 3:1 and N/P ratio of 3 or 4 for siRNA and mRNA respectively.

Formulation type:	siRNA-LNP	mRNA-LNP
Working RNA Concentration Range (mg/mL)*	0.220–0.240	0.170–0.180
Working Buffer Concentration (mM)	25	Minimum 65

**Refer to Appendix A for tabulated RNA concentrations.*

4. Confirm RNA concentrations using a NanoDrop® UV spectrometer, with PNI Formulation Buffer as the reference blank.

RNA-LNP Preparation

Note: *Refer to the the NanoAssemblr Benchtop v1.5 User Guide for more detailed operating instructions.*

1. Set up the NanoAssemblr Benchtop Software with the parameters listed in the table below. Table 1 represents recommended starting parameters.

Note: *Priming pre-loads the NanoAssemblr cartridge with blank buffer and ethanol at the same flow rate ratio (FRR) as the formulation to mitigate unwanted mixing. Steps 1–7 are not required for formulating on the NanoAssemblr Ignite due to an improved fluid injection mechanism.*

Table 1: NanoAssemblr Benchtop Instrument Settings

Volume	2 mL
Flow Rate Ratio	3:1
Total Flow Rate	12 mL/min
Left Syringe Size	3 mL
Right Syringe Size	1 mL
Autoswitch	ON
Start Waste Volume	0.35 mL
End Waste Volume	0.05 mL

2. Insert the NanoAssemblr Benchtop Cartridge into the rotating block.
3. Draw at least 1.5 ml of PNI Formulation Buffer into a 3 mL syringe with help of a blunt needle. Remove air and avoid any bubbles in the syringe tip. Insert the syringe without the needle into the left inlet of the NanoAssemblr Microfluidic Cartridge.
4. Draw at least 0.5 ml of ethanol into a 1 mL syringe using a blunt needle. Remove the air and avoid any bubbles in the syringe tip. Insert the syringe without the needle into the right inlet of the NanoAssemblr Microfluidic Cartridge.
5. Insert sample and waste collection tubes into the respective tube clips.
6. Close the instrument lid, confirm the instrument parameters and press "GO" in the software and confirm the instrument parameters.
7. When the run is complete and the running LED has turned off, open the instrument lid and remove the waste and sample tubes and the syringes.
8. Set up the NanoAssemblr Benchtop Software with the parameters listed in Table 1

Note: *Table 1 lists the recommended starting parameters. These parameters may require optimization to achieve the best RNA encapsulation efficiency and RNA-LNP size, depending on the desired N/P ratio, and characteristics.*

9. Open the lid and insert the cartridge in the cartridge slot. Raise the rotating block until the cartridge Luers are visible.
10. Draw at least 1.5 mL of prepared RNA working solution into a 3 mL syringe. Use a blunt needle if necessary. Remove the needle, clear air bubbles from the syringe and use the plunger to advance the liquid to the tip, avoiding drips from the syringe tip. Insert and twist the syringe into the left inlet of the cartridge to engage the Luer.
11. Draw at least 0.5 mL of prepared GenVoy-ILM working solution into a 1 mL syringe using a blunt needle if necessary. Remove the needle, clear air bubbles from the syringe and use the plunger to advance the liquid to the tip, but avoid drips from the syringe tip. Insert and twist the syringe into the right inlet of the cartridge.
12. Return the rotating block to the downwards position.
13. Mark a 15 mL conical collection tube as "RNA-LNP" and push the left tube holder. Mark another with "waste" and push into the right tube holder.
14. Close the lid and select "GO" on the screen. Confirm the parameters, read and confirm the information in the dialog box, and press "Ok". The pushers are now injecting the fluids into the microfluidic cartridge. The formulation is collected in the tube labeled "RNA-LNP".
15. After the pushers have positioned themselves back in the home position, the running LED will turn off, indicating that it is safe to open the lid. Open the lid and remove the conical collection tube labeled "RNA-LNP" and set aside for characterization and further processing immediately.
16. Raise the rotating block. Remove and discard the syringes. Return the rotating block to the downward position and remove the cartridge.
17. To make additional samples, follow the Routine Cleaning steps in the Benchtop User Guide and then return to Step 1. Repeat Steps 2–16.
18. Measure the size of the particles and their polydispersity index (PDI) by DLS, use an aliquot of the sample and dilute 1:10 in PBS as per the PNI Sizing Protocol.
19. Dilute the prepared particles 40x in sterile Ca^{2+}- and Mg^{2+}-free PBS.
20. Fill the top compartment of an Amicon® Ultra-15 centrifugal filtration tube with the diluted particle solution. Spin the tube at 2000 x g for 30 minutes at 20°C.
21. Remove the solution below the filter unit and repeat as needed until the solution is re-concentrated to the original volume (2 mL).

Note: *To maximize recovery, rinse the centrifugal filtration membrane by gently pipetting the retained sample solution over the membrane several times to dislodge any particles adsorbed to the membrane before recovering or adding additional sample.*

22. Use an Acrodisc® filter (0.2 µm pore size) to sterile-filter the concentrated RNA-LNP sample. Some formulations with larger particles or poor PDI may require prefiltering with 0.45 µm pore size.

Note: *Other sterile filters may interact with RNA-LNPs.*

23. Remeasure the size of your particles and the PDI by DLS as performed in Step 18.
24. Measure the mRNA concentration using the RiboGreen® Assay as per the PNI RiboGreen® protocol (Appendix C). A PNI representative will provide the RNA Quantification Workbook spreadsheet to calculate the RNA concentration.

Related Documents

- NanoAssemblr® Benchtop™ User Guide (PNI-MN-NA-002-EXT)
- RNA Quantification workbook (PNI-WB-S9-001-INT)
- PNI Sizing protocol (PNI-SOP-UN-002-EXT)

Note: *If you did not receive these related documents contact your Field Application Scientist or email us at info@precision-nano.com*

Appendix A - Component Concentration Guidelines

Tables of working concentration of RNA based on working concentration of GenVoy-ILM and the Flow Rate Ratio (FRR).

Important!

This table covers a broad range of possible parameters, many of which have not been validated. The recommended parameters for formulation with GenVoy-ILM are an aqueous:organic Flow Rate Ratio (FRR) of 3:1, and N/P ratio between 3-8, and a GenVoy-ILM working concentration of 12.5 mM.

Considerations when using this table

- Higher N/P ratios generally lead to higher encapsulation efficiencies for larger RNA constructs such as mRNA
- Higher FRR generally leads to smaller nanoparticle size in many formulations

It is important to understand that the chemistry, mixing parameters and concentration of reagents play a significant role in the PDI, size and encapsulation efficiency of your lipid nanoparticle. For more information, please refer to the Resource Center on the PNI website, or contact your local PNI representative.

www.precisionnanosystems.com/resource-center

GenVoy-ILM working concentration (mM)		12.5		25
		RNA working concentration		RNA working concentration
FRR (aq:or)	N/P	mg/mL		mg/mL
1:1	1	2.09		4.17
	2	1.04		2.09
	3	0.70		1.39
	4	0.52		1.04
	5	0.42		0.83
	6	0.35		0.70
	7	0.30		0.60
	8	0.26		0.52
	9	0.23		0.46
	10	0.21		0.42

GenVoy-ILM working concentration (mM)		12.5		25
		RNA working concentration		RNA working concentration
FRR (aq:or)	N/P	mg/mL		mg/mL
2:1	1	2.09		2.09
	2	1.04		1.04
	3	0.70		0.70
	4	0.52		0.52
	5	0.42		0.42
	6	0.35		0.35
	7	0.30		0.30
	8	0.26		0.26
	9	0.23		0.23
	10	0.21		0.21
3:1	1	0.70		1.39
	2	0.35		0.70
	3	0.23		0.46
	4	0.17		0.35
	5	0.14		0.28
	6	0.12		0.23
	7	0.10		0.20
	8	0.09		0.17
	9	0.08		0.15
	10	0.07		0.14
4:1	1	0.52		1.04
	2	0.26		0.52
	3	0.17		0.35
	4	0.13		0.26
	5	0.10		0.21
	6	0.09		0.17
	7	0.07		0.15
	8	0.07		0.13
	9	0.06		0.12
	10	0.05		0.10
5:1	1	0.42		0.83
	2	0.21		0.42
	3	0.14		0.28
	4	0.10		0.21
	5	0.08		0.17
	6	0.07		0.14
	7	0.06		0.12
	8	0.05		0.10
	9	0.05		0.09
	10	0.04		0.08

Appendix B - Syringe compatibility on NanoAssemblr® Ignite™ and Benchtop™

Table of compatible syringes when using GenVoy-ILM with Ignite and Benchtop.

Syringe Compatibility

Syringe	Ignite™	Benchtop™
Becton Dickinson Plastipak™ Luer-Lok™ or Luer Slip (centered tip): 1*, 3, 5, 10 mL	✔	✔
B Braun™ Injekt® Solo Luer Cone or Luer Lock Cone (centered tip): 1, 2, 5, 10 mL	✔	
B Braun™ Omnifix™ Solo Luer Cone or Luer Lock Cone (centered tip): 1, 3, 5, 10 mL	✔	
Terumo® Hypodermic Syringes Luer Lock Tip or Luer Slip Tip (centered tip): 1, 2.5, 3, 5, 10 mL	✔	

** It is not recommended to use 1 mL Becton Dickinson Plastipak™ Luer-Lok™ syringe with GenVoy-ILM*

Appendix C - PNI Ribogreen® Assay Protocol

The following protocol is used to determine the encapsulation efficiency for RNA-LNPs.

Encapsulation Efficiency Assay Protocol for RNA-LNPs

Additional Reagents/Disposables

Description	Recommended Supplier
Quant-iT™ Ribogreen® Assay Kit	Thermo Fisher Scientific
Triton™ X-100	Sigma Aldrich
RNase free water	General Laboratory Supplier
RNase Free Filter Pipette Tips (10, 20, 200, and 1000 µL)	General Laboratory Supplier
Pipette basins	General Laboratory Supplier
96-well clear plate	General Laboratory Supplier
Mg^{2+} / Ca^{2+} free PBS 1x	General Laboratory Supplier
18 Gauge Needles	General Laboratory Supplier

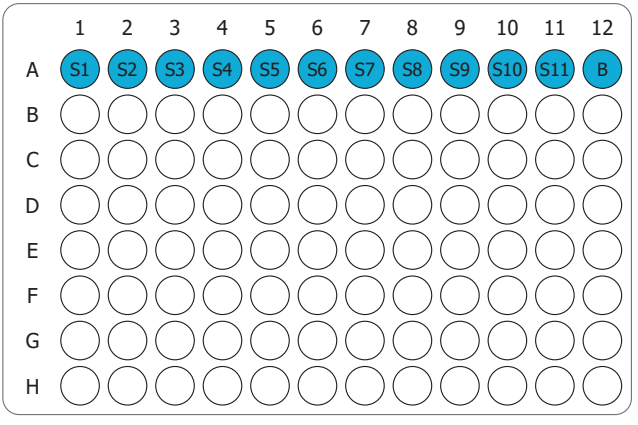
Additional Equipment Required

Description	Recommended Supplier/Product
Plate Reader	Synergy™ H1 Biotek® Plate Reader
Multichannel Pipette (10 – 300 µL)	General Laboratory Supplier
Micropipettes (10, 20, 200, and 1000 µL)	General Laboratory Supplier

Preparation of Sample Stock Solutions

1. Prepare 1X TE buffer from 20X TE buffer by adding 10 mL of 20X TE buffer to 190 mL RNase free water in a clear glass bottle. Shake the bottle to mix.
2. To 100 mL of prepared 1X TE buffer, add 2 mL of Triton X-100. Stir using a magnetic stirrer for 15 min. This solution is the Triton Buffer.
3. Pour the 1X TE buffer and Triton buffer in separate pipette basins.

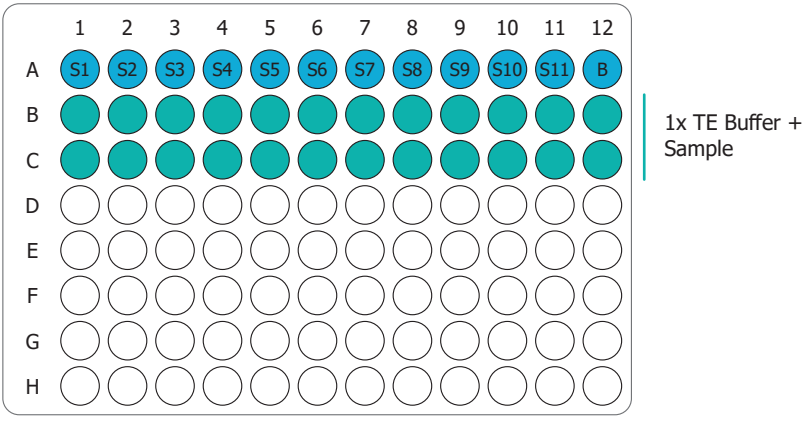
4. In the top row of the 96-well plate (Row A), add 15 µL of sample to these wells (S1-S11). Add 15 µL of PBS to the blank well (B).
5. Using a multi-channel pipette, add 1X TE buffer to Row A to make up the volume to 250 µL. Pipette to mix



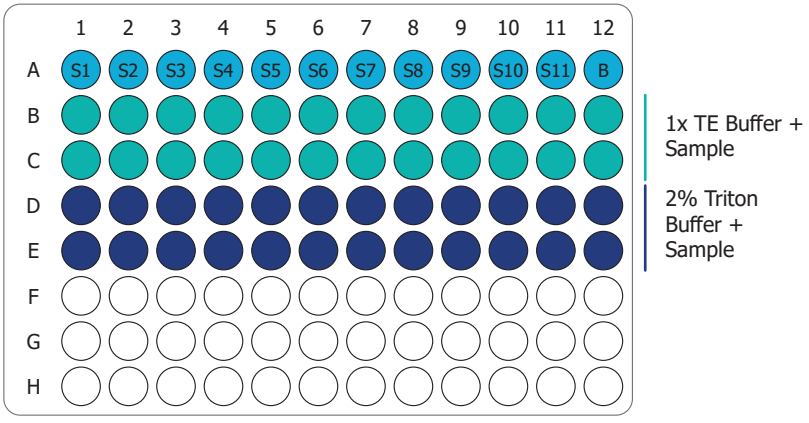
RNA-LNP Sample Setup

This assay is run in duplicate. It is recommended that liquid handling be done using a multi-channel pipette.

1. Add 50 µL of 1X TE buffer to the two wells directly below each sample (Rows B and C).
2. Add 50 µL of sample stock solution from Row A into the wells in Row B and C
3. Add 50 µL of Triton buffer to the wells in Rows D and E below each sample



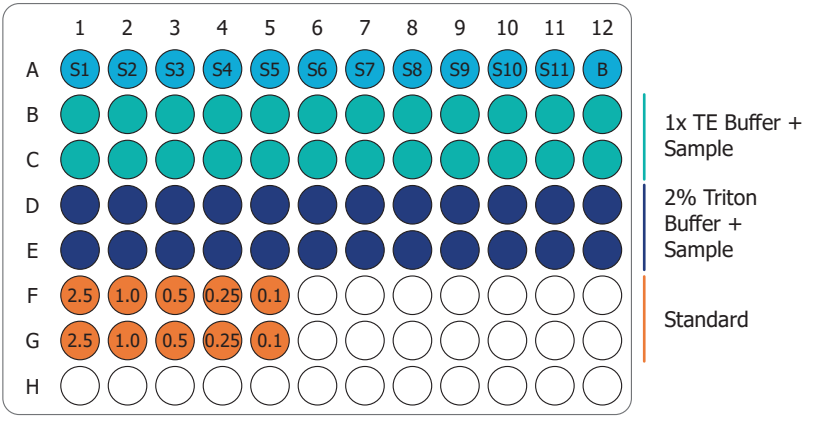
4. Add 50 µL of sample stock solution from Row A into the wells in Rows D and E.



RNA Standard Curve Setup

1. Setup a standard curve (in duplicate in rows F and G) as shown in the table below using the RNA Stock (20 µg/mL RNA), 1X TE Buffer, and Triton Buffer.

Final RNA µg/mL	RNA Stock Required µL	TE Buffer Required µL	Triton Buffer Required µL	Total Volume per Well µL
2.5	25	25	50	100
1	10	40	50	100
0.5	5	45	50	100
0.25	2.5	47.5	50	100
0.1	1	49	50	100



2. Once samples and standard curve are plated, incubate the plate at 37°C for 10 min to lyse the RNA-LNP in the presence of Triton buffer.

Preparation of Ribogreen Solution

1. Sum the total number of sample wells and standard curve wells. Add four to this number, and multiply the total by 100. This is the total volume, in µl, of Ribogreen Solution needed for this assay.
2. In a 15 mL RNase Free Falcon Tube, dilute the Ribogreen Reagent 1:100 into 1X TE buffer to the total volume calculated in the previous step.

   Note: *For example, if 3000 µl of Ribogreen Solution is needed, add 30 µl of Ribogreen Reagent to 2970 µl of 1X TE buffer.*
3. Vortex the Ribogreen Solution for 10s to mix.

Addition of Ribogreen Solution and Sample Reading

1. Remove 96-well plate from 37°C incubator.
2. Add 100 µl of Ribogreen Solution to each well.
3. Pop any bubbles with a needle.
4. Read using fluorescent plate reader with the following settings:

Excitation	485 nm
Emission	528 nm
Optics	Top Read
Gain	55
Read Height	8mm

Note: *The Gain and Read height will change depending on the instrument.*

Sample Analysis

1. Enter each RNA-LNP sample and each Standard Curve sample into the RNA Quantification workbook (PNI-WB-S9-001-INT). This sheet will calculate the encapsulation efficiency and siRNA concentration of each sample.
2. The second sheet on this workbook (Name: Plate Setup) gives the well numbers from which the O.D. values would be fed into the first sheet (Name: RNA Quantification).
3. The third sheet (Name: Dilution factor-calculation) in the workbook gives the calculation to input the dilution factor values in column 'O' of the first sheet (Name: RNA Quantification).

Notes

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