



Nov 21, 2024

mRNA vaccine formulation (Precision Nanosystem)

DOI

<https://dx.doi.org/10.17504/protocols.io.36wgqnn4kgk5/v1>

Carolina Lopez¹

¹Washington University



Carolina Lopez

Washington University

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Protocol Citation: Carolina Lopez 2024. mRNA vaccine formulation (Precision Nanosystem). **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.36wgqnn4kgk5/v1>

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Protocol status: Working

We use this protocol and it's working

Created: May 29, 2024



Last Modified: November 24, 2025

Protocol Integer ID: 100890

Keywords: mrna vaccine formulation, protocol for mrna formulation, mrna formulation, using precision nanosystem reagent, precision nanosystem reagent, precision nanosystem, mrna

Abstract

Protocol for mRNA formulation using Precision Nanosystem reagents and follow up QC

Materials

Required PNI products:



Suggested supplies and equipment:



mRNA vaccine formulation (Precision Nanosystem protocol)

1 RNA and Reagent Preparation

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.



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

2 RNA-LNP Preparation

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



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 the table.
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.
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 PDI Sizing Protocol.

3 Downstream process

30m

1. Dilute the prepared particles 40x in sterile Ca²⁺- and Mg²⁺- free PBS.
2. 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  00:30:00 at  20 °C .
3. Remove the solution below the filter unit and repeat as needed until the solution is re-concentrated to the original volume (2 mL).
4. 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.
5. Remeasure the size of your particles and the PDI by DLS as performed before.

B) Encapsulation efficiency and concentration

25m 10s

- 4 *mRNA concentration and encapsidation efficiency of RNA-LNPs Measure the mRNA concentration using the RiboGreen Assay as per the PNI RiboGreen.*



Additional Equipment Required



5 Preparation of Sample Stock Solutions

15m

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  00:15:00 . 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.



6 RNA-LNP Sample Setup

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.



7 RNA Standard Curve Setup

10m

1. Setup a standard curve (in duplicate in rows F and G) as shown in the table below using the RNA Stock (20 $\mu\text{g}/\text{mL}$ RNA), 1X TE Buffer, and Triton Buffer.



2. Once samples and standard curve are plated, incubate the plate at  37 °C for  00:10:00 to lyse the RNA-LNP in the presence of Triton buffer.

8 Preparation of Ribogreen Solution

10s

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 μ L of Ribogreen Solution is needed, add  30 μ L of Ribogreen Reagent to  2970 μ L of 1X TE buffer.

3. Vortex the Ribogreen Solution for  00:00:10 to mix.

4. Remove 96-well plate from  37 °C incubator.

5. Add  100 μ L of Ribogreen Solution to each well.

6. Pop any bubbles with a needle.

7. Read using fluorescent plate reader with the following settings:

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

9

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).