

GenVoy-ILM reagent

Instructions for Use

Introduction

Read this before unpacking or using the products

This instruction contains information that is important for the safe handling, unpacking, and preparation of the GenVoy-ILM reagent with the PNI formulation buffer.

Before using these products, all users must read this document and the *NanoAssemblr Ignite and Ignite+ Operating Instructions (NIN1134)*.

Intended use

The GenVoy-ILM reagent and the PNI formulation buffer (sold separately) are research use only products for the formulation of RNA-LNPs with a homogenous core structure.

These solutions are supplied as standalone products to use only in combination with the NanoAssemblr™ Ignite or Ignite+ instrument and NxGen™ Ignite cartridges.

The products are intended for research use only and shall not be used in any clinical or *in vitro* procedures for diagnostic or therapeutic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheet (SDS) for each chemical used in the procedure.

Scan the QR code on the packaging to access the SDS for the components.

Background

Description

The GenVoy-ILM reagent is a pre-optimized ionizable lipid mixture (ILM) designed to be used with the PNI formulation buffer to encapsulate mRNA, gRNA, siRNA, miRNA, and tRNA in lipid nanoparticles (LNPs). The products can be used at various stages of RNA medicine development, from discovery to late preclinical.

The GenVoy-ILM reagent is available in two variants: with or without dye.

The reagent and buffer are optimized for preparation of LNPs on a NanoAssemblr Ignite or Ignite+ instrument.

Typical applications

- Deliver RNA *in vitro* in preclinical studies
- Deliver RNA *in vivo* in preclinical studies

Related user resources online

The following resources related to the products can be downloaded from the web.

- *GenVoy-ILM 2 mL and 5 mL Workbook*
- *RNA Quantification workbook*
- *RiboGreen Assay Protocol to Determine RNA Encapsulation Efficiency*

Search for the document name on [cytiva.com](https://www.cytiva.com) to find the files.

Related user documentation

The related user documentation is listed in the table below.

Documentation	Main contents
<i>NanoAssemblr Ignite and Ignite+ Operating Instructions (NIN1134)</i> This document is referred to as <i>the instrument Operating Instructions</i> in this document.	Instructions needed to prepare and operate the Ignite or Ignite+ instrument in a correct and safe way. System overview, site requirements, and instructions for moving the system within the same building. Instructions for basic maintenance and troubleshooting.

Access user documentation online

Scan the QR code or visit [cytiva.com/instructions](https://www.cytiva.com/instructions). Enter the title or the document number to access the file.



General LNP considerations

Overview

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.

N/P ratio

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) that are found on the ionizable lipids and become cationic at low pH, and the phosphates (P) that are found on the RNA backbone and are anionic.

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

Mixing parameters

Among other factors, mixing parameters can influence characteristics such as particle size and polydispersity index (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:

Where,

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

- $[n]$ is the concentration of nucleotides,
- $[L]$ is the lipid mix concentration,
- X is the mole fraction of ionizable cationic lipid,
- FRR is the flow rate ratio, and
- N/P is the N/P ratio.

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.

Product specifications

Introduction

The following sections give the product names, properties, and storage temperatures for the reagent and the formulation buffer.

Note: PNI formulation buffer is sold separately from the GenVoy-ILM reagent. See [Find ordering information online, on page 19.](#)

Product properties

Product	Volume	Concen- tration	Average molecular weight	pH	Excita- tion wave- length	Emis- sion wave- length
GenVoy-ILM	2 mL	25 mM	630.5 g/mol	N/A	N/A	N/A
	5 mL					
GenVoy-ILM with Dye	2 mL	25 mM	631 g/mol	N/A	644 nm	665 nm
	5 mL					
PNI formulation buffer	20 mL	100 mM	N/A	4.0	N/A	N/A

Storage

Product	Storage
GenVoy-ILM	-80°C
GenVoy-ILM with Dye	-80°C, protect from light
PNI formulation buffer	2°C to 8°C

GenVoy-ILM composition

Component	GenVoy-ILM (mol %)	GenVoy-ILM with Dye (mol %)
Ionizable lipid	50	50
Distearoylphosphatidylcholine (DSPC)	10	10
Cholesterol ¹	37.5	37.4
Stabilizer	2.5	2.5
Fluorescent dye (DiD)	0	0.1

¹ Non-animal derived

Required materials

Required materials supplied by Cytiva

- GenVoy-ILM or GenVoy-ILM with Dye (2 mL or 5 mL)
- PNI formulation buffer
- NanoAssemblr Ignite or Ignite+ instrument
- NanoAssemblr Ignite or Ignite+ cartridges
- Sterile syringe filters: modified polyethersulfone (mPES), 13 mm, 0.2 µm, such as Acrodisc™

Note: Other sterile filters can interact with RNA-LNPs.

See [Find ordering information online, on page 19](#) for more information.

Required materials supplied by the user

To prepare RNA-LNPs with the GenVoy-ILM reagent, the following materials are required. All plastic consumables used in the preparation, formulation, and storage of LNPs should be sterile and RNase-free as required.

- Disposable syringes: 1 mL and 10 mL

Note: Refer to the instrument Operating Instructions for guidance on the specific brands that can be used, and the syringe size to use based on the formulation parameters. It is recommended to use the smallest syringe size that will accommodate the required volumes.

- Blunt needles: 18 gauge
- Amicon 10 to 30 kDa molecular weight cut off (MWCO) centrifugal filters, 15 mL
- UV spectrometer
- Refrigerated centrifuge and swing bucket
- Conical centrifuge tubes: 15 and 50 mL
- siRNA or mRNA
- RNase-free phosphate buffered saline (PBS), without calcium and magnesium
- Apolipoprotein-E3 or -E4, if required for primary cell transfection in serum independent media
- Anhydrous ethanol
- 70% ethanol
- RiboGreen Assay materials:
 - Quant-iT RiboGreen Assay Kit
 - Triton X-100

- Invitrogen TE buffer (20X)
- 96-well black bottom plates
- 1X phosphate buffered saline (PBS), without calcium and magnesium

Workflow overview

This section describes the workflow for preparing RNA-LNPs on the Ignite or Ignite+ instrument, including RNA solution preparation, instrument handling, and LNP preparation. Detailed steps for each phase are presented in the following sections.

A typical workflow using GenVoy-ILM reagents is given in the table below.

Phase	Action	Description
1	Fill in the <i>GenVoy-ILM Workbook</i>	Calculate the reagent consumption and material preparation
2	Prepare the workspace and reagents	Thaw and prepare the GenVoy-ILM reagents at working concentrations.
3	Prepare RNA solution	Prepare the RNA solution by combining the RNA payload and the formulation buffer.
4	Formulate RNA-LNPs	Formulate the LNPs on the instrument.
5	Downstream process LNPs	• Perform buffer exchange and concentration of LNPs using centrifugal filters. • Perform sterile filtration.
6	Determine LNP physicochemical characteristics	• Determine the size and polydispersity of LNPs using dynamic light scattering (DLS) or a similar method. • Quantify the encapsulated RNA using the RiboGreen Assay.

Fill in the calculation workbook

The *GenVoy-ILM Workbook* is a tool to calculate parameters and volumes for the LNP formulation on the Ignite and Ignite+ instrument.

The *GenVoy-ILM Workbook* is also referred to as the *Workbook* in this document. The *Workbook* is available online. See [Related user resources online, on page 2](#).

In **Step 1: User Input**, the user must enter values for the parameters in the table below. Recommended values are also given for relevant parameters.

Parameter	Description	Recommended value
Total LNP volume (mL)	Total volume of LNP prepared, including waste Note: <i>This value does not include the volume that is added by in-line dilution.</i>	User determined
Stock RNA concentration (mg/mL)	Concentration of RNA stock solution	As measured using UV-vis
N/P ratio	Molar ratio of amines (nitrogen) in the ionizable lipid to phosphate groups on the nucleic acid	8
GenVoy-ILM concentration (mM)	Concentration of the GenVoy-ILM reagent	User determined Note: <i>The recommended working concentration for the GenVoy-ILM reagent is 12.5 mM, which can be achieved by diluting the stock reagent one-to-one with anhydrous ethanol. The GenVoy-ILM reagent can also be used with a concentration up to the stock concentration.</i>
Flow Rate Ratio C:R	Ratio between the volumes of fluids from the C and R syringes	3

Parameter	Description	Recommended value
Excess solution prepared (%)	Volume of reagent and RNA working solutions prepared above what is required to account for transfer losses.	User determined
Start waste (mL)	Volume of fluid to collect in the waste collection tube at the beginning of the run	Refer to the instrument <i>Operating Instructions</i> for the waste volumes recommended for the syringe sizes used
End waste (mL)	Volume of fluid to collect in the waste collection tube at the end of the run	Refer to the instrument <i>Operating Instructions</i> for the waste volumes recommended for the syringe sizes used

Prepare the workspace and reagents

Follow the steps below to prepare the workspace and the reagents.

Step	Action
1	Clean the work area thoroughly with 70% ethanol.
2	Thaw the GenVoy-ILM lipid mix at 55°C for 5 minutes, in a bead bath or heat block. Keep the tube closed to prevent evaporation when the lipid mix is above room temperature.
3	When the lipid mix is thawed, transfer the tube to room temperature and vortex.
4	Prepare the GenVoy-ILM working solution by diluting the reagent to the target working concentration with anhydrous ethanol: a. Aliquot the volume of GenVoy-ILM reagent (1) indicated in Step 3 of the <i>Workbook</i> into a new tube by reverse pipetting.

Step 3. Required Lipid Mix Volume		
GenVoy-ILM, 25 mM (µL)	138	1
Anhydrous ethanol (µL)	138	2
Total in R (lipid) syringe (µL)	275	
Calculation includes above set excess		

- b.

Add the required volume of anhydrous ethanol (2) to achieve the target concentration, as indicated in Step 3 of the *Workbook*.
- c.

Close the tube immediately to prevent evaporation.
- d.

Vortex the GenVoy-ILM working solution.

Prepare the RNA solution

Introduction

This section describes the procedure to prepare the RNA working solution. There are two different procedures, depending on the type of RNA.

The required RNA concentration depends on GenVoy-ILM concentrations, the flow rate ratio, and the N/P ratio. Detailed information about the impact of different parameters on the concentration recommendations are provided in [Component concentration guidelines, on page 20](#).

When handling RNA solutions, always follow the recommendations below:

- Thaw and keep RNA stock solutions on ice.
- Always open RNA stock solutions in the biosafety cabinet (BSC).
- Aliquot the RNA stock solutions to avoid multiple rounds of freezing and thawing.
- Do not vortex RNA solutions.
- Before use, mix carefully by inversion and gentle pipetting.
- Use RNase-free pipette tips to measure and to transfer RNA solutions.

Prepare the mRNA for one-component delivery

Follow the steps below to prepare the RNA solution. To maintain sterility of the reagents, perform any steps that involve open tubes inside a BSC.

Step	Action
1	Retrieve the frozen RNA aliquot and thaw on ice. Always keep the RNA on ice to prevent material degradation.
2	Once thawed, measure the concentration of the RNA stock solution with UV-vis.

Step	Action
3	Input the measured concentration of the RNA stock solution into Step 1 of the <i>Workbook</i> .

Step 1. User Input	
Total LNP volume (mL)	1.0
Stock RNA concentration (mg/mL)	1
N/P ratio	8
GenVoy-ILM concentration (mM)	12.5
Flow Rate Ratio, C:R	3
Excess solution prepared (%)	10%
Start waste (mL)	0.1
End waste (mL)	0

4	Pipette the volumes of PNI formulation buffer (1) and RNA (2) indicated in Step 2 of the <i>Workbook</i> into a tube for the RNA solution.
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Step 2. RNA Working Solution Preparation	
PNI formulation buffer (μL)	753.3 1
RNA (μL)	71.7 2
Total in C (aqueous) syringe (μL)	825
RNA working solution concentration (mg/mL)	0.087
<i>Calculation includes above set excess</i>	

5	Pipette the sample gently up and down to mix.
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After preparing the RNA solution, confirm the RNA concentrations using a UV spectrometer, with PNI formulation buffer as the reference blank. Keep the RNA stock solution on ice until use.

Prepare the mRNA for two-component delivery

Follow the steps below to prepare the RNA solution. To maintain sterility of the reagents, perform any steps that involve open tubes inside a BSC.

Step	Action
1	Retrieve the frozen RNA aliquot and thaw on ice. Always keep the RNA on ice to prevent material degradation.

- | Step | Action |
|------|--|
| 2 | Once thawed, measure the concentration of the RNA stock solution with UV-vis. |
| 3 | Input the measured concentration of the Cas9 mRNA and sgRNA stock solutions into Step 1 of the <i>Workbook</i> . |

Step 1. User Input	
Total LNP volume (mL)	1.0
Stock Cas9 mRNA concentration (mg/mL)	1
Stock sgRNA concentration (mg/mL)	1
Cas9 / sgRNA ratio (wt.)	1
N/P ratio	8
GenVoy-ILM concentration (mM)	12.5
Flow Rate Ratio, C:R	3
Excess solution prepared (%)	10%
Start waste (mL)	0.1
End waste (mL)	0

- 4 Pipette the volumes of PNI formulation buffer (1), Cas9 mRNA (2), and sgRNA (3) indicated in Step 2 of the *Workbook* into a tube for the RNA solution.

Step 2. RNA Working Solution Preparation	
PNI formulation buffer (μL)	753.3 ①
Cas9 mRNA (μL)	35.9 ②
sgRNA (μL)	35.9 ③
Total in C (aqueous) syringe (μL)	825
RNA working solution concentration (mg/mL)	0.087
<i>Calculation includes above set excess</i>	

- 5 Pipette the sample gently up and down to mix.

After preparing the RNA solution, confirm the RNA concentrations using a UV spectrometer, with PNI formulation buffer as the reference blank. Keep the RNA stock solution on ice until use.

Formulate RNA-LNPs

The Ignite instrument can prepare formulation volumes of 1 to 20 mL. The following sections give instructions on how to prepare an RNA-LNP formulation on the Ignite or Ignite+ instrument.

Step	Action
1	Turn on the instrument as described in the instrument <i>Operating Instructions</i> .
2	Wipe down the instrument with RNase decontamination solution and 70% isopropanol.
3	On the main menu, tap Quick Run .
4	Input the calculated parameters as given in Step 1 of the <i>Workbook</i> . Refer to the instrument <i>Operating Instructions</i> for details.
5	Insert the cartridge adapter and cartridge into the instrument according to the instrument <i>Operating Instructions</i> .
6	Prepare the C syringe with the RNA working solution: a. Draw the total amount of RNA solution into the C syringe as indicated in Step 2 of the <i>Workbook</i>, using a clean blunt needle if necessary. b. Remove the needle from the syringe. c. Clear any air bubbles from the syringe. d. Use the plunger to advance the liquid in the syringe, making sure to avoid drips from the tip of the syringe.
7	Prepare the R syringe with the GenVoy-ILM working solution: a. Vortex the GenVoy-ILM working solution. b. Draw the total amount of GenVoy-ILM solution into the R syringe as indicated in Step 3 of the <i>Workbook</i>, using a clean blunt needle if necessary. c. Remove the needle from the syringe. d. Clear any air bubbles from the syringe. e. Use the plunger to advance the liquid in the syringe, making sure to avoid drips from the tip of the syringe.

Step	Action
8	Continue to attach the R syringe and the C syringe and begin the formulation procedure on the instrument as described in the instrument <i>Operating Instructions</i>. Note: Use two 15 mL conical centrifuge tubes to collect the sample and the waste.

When the formulation is finished, set aside the RNA-LNP sample for characterization and further processing immediately. To make additional samples, return to the **Quick Run** screen and repeat the steps above.

Downstream process the LNPs

Follow the steps below after the formulation on the instrument is complete.

Note: *The steps and materials listed below are an example and can be adjusted based on the formulation and customer-specific process needs.*

Step	Action
1	Characterize the RNA-LNP sample: a. Aliquot the sample and dilute the aliquot to 1:10 in PBS. b. Measure the size of the particles and their PDI by dynamic light scattering (DLS). Contact Cytiva for a detailed sizing protocol, if needed.
2	Dilute the RNA-LNP sample 40X with PBS.
3	Fill the centrifugal filters with the diluted RNA-LNP solution.
4	Spin at 2000 x g for 20 minutes at 4°C in a swing bucket rotor.
5	Discard the solution below the filter unit and repeat step 3 and step 4 as necessary until the entire sample is re-concentrated to approximately the starting RNA-LNP volume (2 mL).
	Tip: <i>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.</i>
6	Inside a BSC, filter the sample using an Acrodisc 0.2 µm sterile filter into a sterile tube.
	Note: <i>Some formulations with larger particles or poor PDI might require prefiltering with 0.45 µm pore size.</i>
7	Re-measure the size of the particles by DLS.
8	Determine the RNA concentration of the LNPs with the RiboGreen Assay. Refer to <i>RiboGreen Assay Protocol to Determine RNA Encapsulation Efficiency</i> , for detailed instructions. See Related user resources online, on page 2 .

Find ordering information online

The latest information about product offerings and product codes is available online. Follow the steps below to find lipid nanoparticle formulation systems, reagents, cartridges, or other accessories.

Step	Action
1	Navigate to cytiva.com .
2	Search for the product name or product category.
3	Navigate to the relevant product page.
4	Scroll to Product Specifications to find the product names, codes, and other ordering information.

Component concentration guidelines

The following table lists the working concentration of RNA, based on the working concentration of GenVoy-ILM and the FRR. This table covers a broad range of possible parameters, many of which have not been validated.

Note: *The recommended parameters for formulation with GenVoy-ILM are:*

- *Working concentration of 12.5 mM*
- *Aqueous:organic flow rate ratio of 3:1*
- *N/P ratio from 3 to 8*

The chemistry, mixing parameters, and concentration of reagents play a significant role in the PDI, size, and encapsulation efficiency of the formulated lipid nanoparticle. For further information, contact your local Cytiva representative.

Consider the following 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.

FRR	N/P ratio	GenVoy-ILM working concentration	
		12.5 mM	25 mM
		RNA working concentration (mg/mL)	
1:1	1	2.086	4.173
	2	1.043	2.086
	3	0.695	1.391
	4	0.522	1.043
	5	0.417	0.835
	6	0.348	0.695
	7	0.298	0.596
	8	0.261	0.522
	9	0.232	0.464
	10	0.209	0.417
2:1	1	1.043	2.086
	2	0.522	1.043
	3	0.348	0.695
	4	0.261	0.522
	5	0.209	0.417

FRR	N/P ratio	GenVoy-ILM working concentration	
		12.5 mM	25 mM
		RNA working concentration (mg/mL)	
	6	0.174	0.348
	7	0.149	0.298
	8	0.130	0.261
	9	0.116	0.232
	10	0.104	0.209
3:1	1	0.695	1.391
	2	0.348	0.695
	3	0.232	0.464
	4	0.174	0.348
	5	0.139	0.278
	6	0.116	0.232
	7	0.099	0.199
	8	0.087	0.174
	9	0.077	0.155
	10	0.070	0.139
4:1	1	0.522	1.043
	2	0.261	0.522
	3	0.174	0.348
	4	0.130	0.261
	5	0.104	0.209
	6	0.087	0.174
	7	0.075	0.149
	8	0.065	0.130
	9	0.058	0.116
	10	0.052	0.104
5:1	1	0.417	0.835
	2	0.209	0.417
	3	0.139	0.278
	4	0.104	0.209
	5	0.083	0.167
	6	0.070	0.139

FRR	N/P ratio	GenVoy-ILM working concentration	
		12.5 mM	25 mM
		RNA working concentration (mg/mL)	
	7	0.060	0.119
	8	0.052	0.104
	9	0.046	0.093
	10	0.042	0.083

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