Cytiva™ RNA delivery LNP kit

Instructions for Use

Original instructions

Introduction

Read this before unpacking or using the kit

This instruction contains information that is important for the safe handling, unpacking, and preparation of Cytiva™ RNA delivery LNP kit, 3 mL and 6 mL.

Before using this product, all users must read this document and the *NanoAssemblr™ Ignite™ and Ignite+™ Operating Instructions*.

Intended use

Cytiva RNA delivery LNP kit is a research use only kit for the delivery of RNA in vaccine development.

This kit is supplied as a standalone product to use only in combination with the Ignite or Ignite+ instrument and NxGen™ Ignite cartridges.

The product is 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 Cytiva RNA delivery LNP kit is a lipid nanoparticle (LNP) reagent mix optimized for the delivery of RNA in preclinical vaccine applications using small murine models, available in two sizes: 3 mL and 6 mL. The Cytiva RNA delivery LNP kit includes an ionizable lipid mix and all the required reagents to prepare LNPs on a NanoAssemblr Ignite or Ignite+ instrument.

Typical applications

- *In vivo* delivery of mRNA and saRNA in preclinical vaccine studies
- *In vitro* RNA payload screening

Related user resources online

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

- *Cytiva RNA delivery LNP kit Workbook*
- *RiboGreen Assay Protocol to Determine RNA Encapsulation Efficiency*

Search for the document name on *cytiva.com* to find the files.

Related user documentation

The related user documentation is listed in the table below.

Documentation	Main contents
NanoAssemblr Ignite and Ignite+ Operating Instructions (NIN1134) This document is referred to as *the instrument Operating Instructions* 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 trouble-shooting.

Access user documentation online

Scan the QR code or visit *cytiva.com/instructions*. Enter the title or the document number to access the file.

Kit components

Components and storage

The following table gives the component names and storage temperatures for all kit components.

Label	Content	Size	Storage
Lipid mix	Lipid mix	3 mL	-80°C
		6 mL	
Formulation buffer type 1	Formulation buffer	6 mL	2°C to 8°C
Dilution buffer type 1 10X	Dilution buffer	100 mL	2°C to 8°C
Cryopreservation buffer type 1	Cryopreservation buffer	12 mL	-80°C

Note: *All kit components are shipped under dry ice and might arrive frozen. Before use, make sure the buffers are fully thawed, and have been re-solubilized by vortex mixing for 30 seconds.*

Expiry

See individual components.

Kit capacity

At an N/P ratio[1] of 8, the kits can formulate the following amounts of RNA:

Kit	Formulation amount
Cytiva RNA delivery LNP kit, 3 mL	~0.7 mg RNA
Cytiva RNA delivery LNP kit, 6 mL	~1.5 mg RNA

When a total volume of 2 mL LNP is prepared with a flow rate ratio (FRR) of 3:1 and N/P ratio of 8, a typical RNA recovery of 70% to 80% is expected in the final sample.

When a lower N/P ratio is used to formulate LNPs, the RNA input is proportionally larger.

[1] N/P ratio represents the molar ratio of amines (nitrogen) in the ionizable lipid to phosphate groups on the nucleic acid.

Required materials

Required materials supplied by Cytiva

- NanoAssemblr Ignite or Ignite+ instrument
- NanoAssemblr Ignite or Ignite+ cartridges

See *Find ordering information online, on page 20* for more information.

Required materials supplied by the user

To prepare RNA-LNPs with the kit, 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.

- Fluorescence plate reader
- Heating block or oven capable of heating to 55°C
- UV spectrometer
- Vortex mixer
- Refrigerated centrifuge and swing bucket
- Conical centrifuge tubes: 15 and 50 mL
- 0.5 to 2 mL tubes with screw cap and O-ring seal
- Disposable syringe: 1, 3, 5, or 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.*
- Micropipettes and pipette tips
- Pipet aid and serological pipets
- Blunt needles
- Sterile syringe filters: 13 mm, 0.2 µm, polyethersulfone (PES)
- Amicon® 30 kDa molecular weight cut off (MWCO) centrifugal filters,15 mL
- 96-well black bottom plates, non-treated surface
- Quant-iT RiboGreen Assay Kit
- Triton X-100
- Molecular biology grade water
- 70% isopropanol
- RNase decontamination solution

Workflow overview

This section describes the workflow for preparing RNA-LNPs on the Ignite and Ignite+ instrument, including RNA solution preparation, instrument handling, and LNP preparation. A typical workflow using a Cytiva RNA delivery LNP kit is given in the table below.

Step	Action	Description
1	Fill in the *Cytiva RNA delivery LNP Workbook*	Calculate the reagent consumption and material preparation
2	Prepare the workspace and reagents	Thaw and prepare reagents at working concentrations
3	Prepare RNA solution	Prepare the RNA solution by combining the RNA payload and the included formulation buffer and molecular biology grade water
4	Formulate RNA-LNPs	Formulate the LNPs on the instrument
5	Downstream process LNPs	• Perform buffer exchange and concentration of LNPs using centrifugal filters • Add cryopreservation buffer • 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
7	Dilution to dosing concentration	Based on the encapsulated RNA concentration, dilute the RNA-LNPs to the dosing concentration

Fill in the calculation workbook

The *Cytiva RNA delivery LNP kit Workbook* is a tool to calculate parameters and volumes for the LNP formulation on the Ignite and Ignite+ instrument.

The *Cytiva RNA delivery LNP kit 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 flow rate (mL/min)`	Sum of the flow rates of the C and R channels, before the optional in-line dilution	20 mL/min
`Total LNP volume (mL)`	Total volume of LNP prepared, including waste ***Note:*** *This value does not include the volume that is added by in-line dilution.*	User determined
`RNA stock concentration (mg/mL)`	Concentration of RNA stock solution	As measured using UV-vis
`Lipid mix working concentration (ratio of stock concentration)`	Concentration of the lipid mix used in the procedure in relation to the stock lipid mix provided in the kit	1
`In-line dilution ratio Dil:LNP (X:1)`	Ratio of diluent:LNP if in-line dilution is used	2 ***Note:*** *Leave blank, or enter* `N/A` *if not applicable*
`Flow rate ratio Aq:Lipid (X:1)`	Ratio between the volumes of fluids from the C and R syringes	3
`N/P ratio`	Molar ratio of amines (nitrogen) in the ionizable lipid to phosphate groups on the nucleic acid	8

Parameter	Description	Recommended value
`Lipid mix excess (mL)`	Volume of lipid mix prepared above what is required to account for transfer losses.	User determined
`RNA solution excess (mL)`	Volume of RNA solution 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 *Operating Instructions* 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 *Operating Instructions* for the waste volumes recommended for the syringe sizes used

Note: *For larger, more complex RNA structures, the following formulation and process parameters are recommended to produce LNPs with a smaller size, lower polydispersity index (PDI), and higher RNA encapsulation:*

- *N/P ratio of 8*
- *Flow rate ratio (FRR) of 5*
- *Lipid mix working concentration of 0.5X*

Prepare the workspace and reagents

Follow the steps below to prepare the workspace and the reagents. To keep materials sterile, perform all steps inside a biosafety cabinet (BSC) and use sterile plastic labware.

Step	Action
1	Turn on and clean the BSC by wiping it down with RNase decontamination solution followed by 70% isopropanol.
2	Thaw the lipid tube 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. ***Note:*** *After the lipid mix is first thawed, it is recommended to aliquot the lipid mix into smaller volumes. This helps maintain sterility and reduce the number of freeze thaw cycles.*
4	Place the following items in the BSC: • The kit components: lipid mix, formulation buffer, dilution buffer, cryopreservation buffer • Molecular biology grade water • Sterile tubes of the appropriate volume for the preparation of the working concentration solutions: - Dilution buffer - 2X cryopreservation buffer - 1X cryopreservation buffer - RNA solution - Lipid mix • Sterile tubes labeled appropriately for the following samples: - Concentrated sample: a collection tube for the RNA-LNP sample prior to sterile filtration - Cryopreservation sample: a collection tube to dilute RNA-LNP sample with cryopreservation buffer prior to sterile filtration - Sterile sample: a collection tube for the final RNA-LNP after sterile filtration

Step	Action

- Analysis sample: a collection tube to aliquot a small volume of LNPs for dynamic light scattering (DLS) and RiboGreen assay

Note:
The volume and quantity of each type of tube depends on the formulation parameters.

- 20, 200 µL, and 1 mL micropipettes and RNase-free pipette tips
- RNase-free serological pipets (of the required volumes and quantity) and pipet aid
- Syringes, needles and 0.2 µm filters
- Ethanol, if needed

5 Prepare the 1X dilution buffer in a sterile tube:

a. Input the required final volume of the 1X dilution buffer in Step 2 in the *Workbook*.

Step 2. Dilution buffer Preparation	
Dilution buffer type 1 10X (mL)	2.0
Molecular biology grade water (mL)	18.0
Final volume 1X Dilution buffer (mL)	20.0

b. Dilute the 10X dilution buffer provided in the kit using molecular biology grade water according to the volumes listed in Step 2.

6 Prepare the 2X cryopreservation buffer in a sterile tube:

a. Input the required final volume of 2X cryopreservation buffer in Step 3 in the *Workbook*.

Step 3. 2X Cryopreservation buffer Preparation	
Cryopreservation buffer type 1 (5X) (mL)	2.00
Molecular biology grade water (mL)	3.00
Final volume 2X Cryopreservation buffer (mL)	5.00

b. Dilute the 5X cryopreservation buffer provided in the kit using molecular biology grade water according to the volumes listed in Step 3.

7 Prepare the 1X cryopreservation buffer in a sterile tube:

Step	Action

a. Input the required final volume of the 1X cryopreservation buffer in Step 4 in the *Workbook*.

Step 4. 1X Cryopreservation buffer Preparation	
Cryopreservation buffer type 1 (5X) (mL)	1.00
Molecular biology grade water (mL)	4.00
Final volume 1X Cryopreservation buffer (mL)	5.00

b. Dilute the 5X cryopreservation buffer provided in the kit using molecular biology grade water according to the volumes listed in Step 4.

Tip:
For the most precise volume calculation, wait to perform this step until immediately before beginning the dilution procedure in *Dilute to dosing concentration, on page 19**.*

8 Prepare the lipid mix as follows:

a. Make sure the lipid mix is cooled to room temperature.

b. Vortex the tube before use to make sure that the lipid mixture is homogenous.

c. Add the required volume of lipid mix (1) to a sterile tube of the appropriate size, according to Step 5 of the *Workbook*. If using dilution, add the volume of ethanol (2) to the tube and mix well by pipetting.

Step 5. Lipid mix Preparation		
Lipid mix (mL)	0.825	1
Ethanol (mL)	0.000	2
Final volume Lipid mix (mL)	0.825	

Prepare RNA solution

When handling RNA solutions, always follow the recommendations below:

- Thaw and keep RNA stock solutions on ice.
- Always open RNA stock solutions in the 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.

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.
3	Input the measured concentration of the RNA stock solution into Step 1 of the *Workbook*.

Step 1. User Input	
Total flow rate (mL/min)	20
Total LNP volume (mL)	2.5
RNA stock concentration (mg/mL)	1
Lipid mix working concentration (ratio of stock concentration)	1
In-line dilution ratio Diluent:Aq+Lipid (X:1)	N/A
Flow rate ratio Aqueous:Lipid (X:1)	3
N/P ratio	8
Lipid mix excess (mL)	0.2
RNA solution excess (mL)	0.3
Start waste (mL)	0.45
End waste (mL)	0.05

4	Pipette the volumes of formulation buffer (1) and molecular biology grade water (2) indicated in Step 6 of the *Workbook* into a tube for the RNA solution.	

Step 6. RNA solution Preparation		
Formulation buffer type 1 (µL)	218	1
Molecular biology grade water (µL)	1790	2
RNA (µL)	168	3
Final volume RNA solution (µL)	2175	

Step	Action
5	Add the required volume of the RNA (3) to the RNA solution tube, as indicated in Step 6 of the *Workbook*.
6	Pipette the sample gently up and down to mix.

Keep the RNA stock solution on ice until use.

Formulate RNA-LNPs

Follow the steps below to formulate the RNA-LNPs on the Ignite or Ignite+ instrument.

Step	Action
1	Turn on the instrument as described in the instrument *Operating Instructions*.
2	Wipe down the instrument with RNase decontamination solution followed by 70% isopropanol.
3	On the main menu, tap ***Quick Run***.
4	Select the syringes to be used for **L**, **C**, and **R** solutions based on the calculated volumes required for each solution, shown in grey in Step 7 of the *Workbook*. ***Note:*** *It is recommended to use the smallest syringe size that will accommodate the calculated volumes.*

Step 7. Ignite Parameters	
L (Diluent) syringe **volume** (mL)	N/A
C (Aqueous) syringe **volume** (mL)	1.88
R (Lipid) syringe **volume** (mL)	0.63
Flow rate ratio C:R	3:1
Total volume (mL)	2.5
Total flow rate (mL/min)	20
Dilution ratio L:(C+R)	N/A
Start waste (mL)	0.45
End waste (mL)	0.05

5	On the instrument user interface, enter the run parameters shown in the cells in orange in Step 7 of the *Workbook*. Refer to the instrument *Operating Instructions* for details.
6	Insert the cartridge into the instrument.
7	Prepare the **R** syringe with the lipid mix: **a.** Vortex the lipid mix solution. **b.** Draw the required amount of lipid mix into the syringe using a clean blunt needle. ***Note:*** *It is recommended to draw 100 to 200 µL excess into the syringes to make sure that the full volume can be pushed through the cartridge.* ***Tip:*** *To prevent air bubbles, follow the steps below:*

Step	Action
	i. Draw 0.2 to 0.3 mL into the syringe, clear of the needle. *ii. Clear any air bubbles near the rubber gasket by tapping the syringe.* *iii. Dispense the air in the syringe carefully until a drop is seen at the end of the needle.* *iv. Draw the remaining solution into the syringe, clearing the needle.* **c.** Remove the needle 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. **e.** Make sure the required volume plus a small excess is present in the **R** syringe.
8	Draw the entire solution from the RNA solution tube into the **C** syringe using the process described above for the **R** syringe. ***Note:*** *Do not vortex the RNA solution. Instead, pipette the solution gently up and down to mix.*
9	If the procedure includes in-line dilution, draw the 1X dilution buffer into the **L** syringe using the process described above for the **R** syringe.
10	Continue to attach the **R** syringe, **C** syringe, and **L** syringe (if needed) and begin the formulation procedure on the instrument as described in the instrument *Operating Instructions*.

Downstream process the LNPs

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

Step	Action
1	Dilute the RNA-LNPs with the volume of 1X dilution buffer given in Step 8 in the *Workbook*, so that a total 10-fold dilution is performed. ***Note:*** *For example, if a 2:1 in-line dilution was performed (3-fold in-line dilution), the workbook calculates the volume such that a manual 3.33-fold dilution with 1X dilution buffer is subsequently performed.*

Step 8. Downstream Processing	
Formulated LNP volume (mL)	2
Volume of 1X Dilution buffer to add (mL)	18
Concentrated LNP volume post-centrifugal filtration (mL)	2.00
Final theoretical RNA concentration (µg/mL)	36.1

2	Keep the diluted RNA-LNPs on ice until centrifugal filtration.
3	Fill the centrifugal filters with the diluted RNA-LNPs.
4	Spin at 2000 x g for 20 minutes at 4°C in a swing bucket rotor. ***Note:*** *Higher speeds reduce the processing time. However, caution is required with RNA structures that are longer and more complex. The maximum recommended speed is 4000 x g.*
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.
6	Perform a 5-fold dilution with 1X dilution buffer in the centrifugal filter, and re-concentrate the sample to the starting RNA-LNP volume.
7	Discard the solution below the filter unit.
8	Repeat *step 6* and *step 7*. When re-concentrating, target a volume of approximately half the desired RNA-LNP volume.

Step	Action
9	Recover the sample from the filter using a micropipette and transfer it into the tube for the concentrated sample. ***Note:*** *It is recommended to pipette the sample over the membrane to improve recovery.*
10	Optional. Wash the filter membrane with approximately 100 to 200 µL of 1X dilution buffer to increase the recovery of RNA-LNPs.
11	Measure the volume of RNA-LNP recovered from the centrifugal filter, including the wash volume. Input the value in Step 8 of the *Workbook* (1). **Step 8. Downstream Processing** Formulated LNP volume (mL): 2 Volume of 1X Dilution buffer to add (mL): 18 Concentrated LNP volume post-centrifugal filtration (mL): 2.00 (1) Final theoretical RNA concentration (µg/mL): 36.1 (2) The workbook calculates the theoretical concentration of the final RNA-LNP sample after dilution with cryopreservation buffer and sterile filtration (2).
12	Pipette an equal volume of the sample and the 2X cryopreservation buffer into the tube for the cryopreservation sample.
13	Pipette the sample gently up and down at least five times to mix the LNPs with the buffer.
14	In the BSC, filter the cryopreservation sample using a sterile 0.2 µm filter into the sterile tube for the sterile sample. ***Tip:*** *To maximize LNP recovery, push air through the filter two to three times to make sure all LNP residues are collected.*
15	Aliquot the required volume of the RNA-LNPs into the tube for the analysis sample.
16	Store the sterile sample tube under refrigerated conditions while awaiting results from the physicochemical analysis.
17	Measure the size of the particles by dynamic light scattering (DLS). Contact Cytiva for a detailed sizing protocol, if needed.

Step	Action
18	Determine the RNA concentration of the LNPs with the RiboGreen Assay. Refer to *RiboGreen Assay Protocol to Determine RNA Encapsulation Efficiency*, available on the web, for detailed instructions. See *Related user resources online, on page 2*.

Dilute to dosing concentration

Perform the following steps inside the BSC to maintain the sterility of the RNA-LNP sample or samples.

Step	Action
1	Dilute the RNA-LNPs to the dosing concentration with sterile 1X cryopreservation buffer. ***Note:*** *Use the results from the RiboGreen assay to determine the volumes required to achieve the dosing concentration.*
2	Mix well by gently pipetting up and down at least five times.
3	Aliquot the sample into multiple tubes to limit the number of freeze-thaw cycles to which the RNA-LNPs are subjected. ***Note:*** *No more than one freeze-thaw cycle is recommended per sample.*

The RNA-LNPs are now ready for use. For short-term storage, store the sample at 4°C and use within 1 week. For long-term storage up to 9 months, store the samples at -80°C.

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.

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