PRECISION NANOSYSTEMS
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Cytiva™ CD34+ HSC LNP kit, 2 mL

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™ CD34+ HSC LNP kit, 2 mL.

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

Intended use

Cytiva CD34+ HSC LNP kit, 2 mL is intended for the delivery of RNA into stimulated human hematopoietic stem cells (HSCs).

This kit is supplied as a standalone product to use only in combination with the 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

Cytiva CD34+ HSC LNP kit, 2 mL is a lipid nanoparticle (LNP) reagent mix optimized for the delivery of RNA (such as Cas9 mRNA/sgRNA) into stimulated human HSCs.

The Cytiva CD34+ HSC LNP kit includes reagents that are used with the NanoAssemblr Ignite or Ignite+ instrument to produce RNA-LNPs.

This non-viral delivery method can be integrated into standard HSC culture workflows. The Cytiva CD34+ HSC LNP kit enables researchers to establish a clinically relevant and scalable method at the preclinical scale for *ex vivo* gene delivery and editing. The kit is suitable for use in the development of gene-modified HSC therapies.

Typical applications

- Deliver mRNA to express proteins of interest
- Deliver nuclease mRNA for genetic engineering, such as CRISPR/Cas gene knockout
- Deliver siRNA for transient gene silencing

Related products

For discovery and research scale applications with the NanoAssemblr Spark™ instrument, the following products can be used.

Product	Product code
Cytiva CD34+ HSC LNP kit, 100 µL	1003000
Cytiva CD34+ HSC LNP kit, 100 µL with cartridges	1004000
NanoAssemblr Spark instrument	NIS0001

Related documentation

Document	Document ID
NanoAssemblr Ignite and Ignite+ Operating Instructions	NIN1134
Cytiva CD34+ HSC LNP kit, 2 mL Workbook	hsckitmrnaignite-WB-1223
RiboGreen Assay Protocol to Determine RNA Encapsulation Efficiency	PNI-WB-S9-001-INT
Genome Editing of CD34+ Hematopoietic Stem and Progenitor Cells with Lipid Nanoparticles	HSC-AN-1123

Access user documentation online

Scan the QR code or visit *precisionnanosystems.com/instructions*.

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	2 mL	-80°C
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 2	Cryopreservation buffer	6 mL	-80°C
Apolipoprotein-E3 (ApoE3)	Apolipoprotein-E3 (ApoE3)	500 µg	-80°C

Expiry

See individual component packaging.

Kit capacity

Up to 5 mL of lipid nanoparticle formulation, or approximately 1 mg of input RNA. For example:

- 5 x 1 mL LNPs
- 1 x 5 mL LNPs

Required materials

Required materials supplied by Precision NanoSystems

Product	Product code
NanoAssemblr Ignite or Ignite+ instrument	Ignite: NIS0001 Ignite+: 1001413
NanoAssemblr NxGen Ignite cartridges	100 pack: NIN0061 200 pack: NIN0062

Required materials supplied by the user

To prepare RNA-LNPs with the Cytiva CD34+ HSC LNP kit, the following equipment, consumables, and reagents are required.

- Fluorescence plate reader
- Heating block or oven capable of heating to 55°C
- Oven, capable of heating to 37°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
- Micropipettes and RNase free pipette tips – 10, 20, 200 and 1000 µL
- Blunt needles
- Syringe filters – 13 mm, 0.2 µm, polyethersulfone (PES)
- Amicon® 30 kDa molecular weight cut off (MWCO) centrifugal filters, 15 mL

  Note: *One centrifugal filter per 1.5 mL of undiluted LNPs (45 mL diluted) is recommended. The use of additional filters does not reduce recovery but saves downstream processing time.*
- 96-well black bottom plates
- Quant-iT RiboGreen Assay Kit
- Triton X-100
- 1X phosphate buffered saline (PBS), without calcium and magnesium
- Molecular grade water (RNase/DNase and endotoxin-free)
- 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 CD34+ HSC LNP kit is given in the table below.

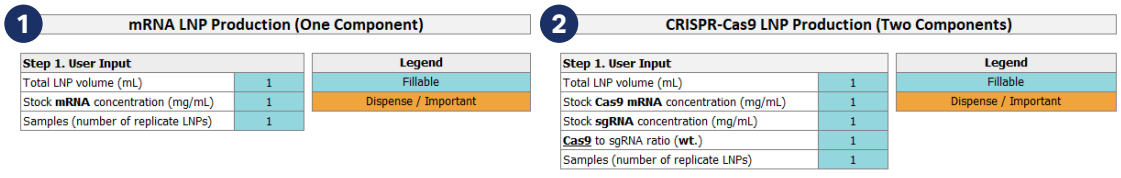
Phase	Action	Description
1	Fill in the *Cytiva CD34+ HSC LNP kit, 2 mL Workbook*	Calculate the reagent consumption and material preparation
2	Prepare the workspace and reagents in a biosafety cabinet	Thaw reagents and materials, label vials, and set up the instrument
3	Prepare RNA solution	Prepare the RNA working solution by combining the RNA payload and the included formulation buffer
4	Formulate the RNA-LNPs	Formulate the LNPs on the instrument
5	Downstream process the LNPs	Clean up the formulation by buffer exchanging using an MWCO centrifugal filter
6	Quantify loaded RNA	Quantify the encapsulated RNA using the RiboGreen Assay
7	LNP treatment of HSCs	Add the RNA-LNPs to the cells

Fill in the calculation workbook

The *Cytiva CD34+ HSC LNP kit, 2 mL Workbook* is a tool to calculate parameters and volumes for the LNP formulation on the Ignite and Ignite+ instrument.

The *Cytiva CD34+ HSC LNP kit, 2 mL Workbook* is also referred to as the *Workbook* in this document. The *Workbook* is available on the web. See *Access user documentation online, on page 3*.

The workbook is divided into two sections to account for the two applications listed below, and the user must only fill in the inputs under the relevant section header:



1. For mRNA expression
2. For CRISPR Cas9 editing (with Cas9 mRNA and sgRNA)

In Step 1, the user must enter values for the parameters given below. Recommended parameter values are listed in the table.

Parameter	Description	Recommended value
Total LNP volume (mL)	Volume of LNP formulation resulting from the procedure	Greater than 0.5 mL
Stock mRNA concentration (mg/mL)[1]	Concentration of mRNA stock solution	User determined
Stock Cas9 mRNA concentration (mg/mL)[2]	Concentration of Cas9 mRNA stock solution	User determined
Stock sgRNA concentration (mg/mL)[2]	Concentration of sgRNA stock solution	User determined ***Note:*** *A commonly used sgRNA stock concentration is 100 µM or roughly 3.2 mg/mL for synthetic constructs.*
Cas9 to sgRNA ratio (wt.)[2]	The weight ratio of Cas9 mRNA to sgRNA	1:1
Samples (number of replicate LNPs)	The number of LNP sample replicates	User determined

[1] For mRNA expression only
[2] For CRISPR Cas9 editing only

The optimized calculations presented in the *Workbook* use a 12 mL/min total flow rate, 2:1 flow rate ratio, and 10% start waste volume.

Prepare the workspace and the reagents

It is important to keep all materials sterile and conduct all work within the biosafety cabinet (BSC). Hematopoietic stem cells are especially sensitive to pyrogens, such as endotoxins, even at minimally detectable levels.

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

Step	Action
1	Turn on and clean the BSC by wiping it down with 70% isopropanol and RNase decontamination solution.
2	Retrieve the frozen RNA aliquot or aliquots and thaw on ice. Always keep the RNA on ice to prevent material degradation.
3	Thaw the lipid mix at 55°C for 5 minutes in a bead bath or heat block. After thawing, keep the lipid mix at room temperature in the BSC. Keep the vial closed to prevent evaporation.

Step	Action
4	Vortex the lipid mix tube to ensure homogeneity. Spin down in a microcentrifuge for 3 to 5 seconds.
5	Place the following items in the BSC:

On ice:	At room temperature:
• RNA aliquot(s)	• One 15 mL conical tube for each LNP sample and one extra for waste • The kit components: lipid mix, formulation buffer, and dilution buffer • Four tubes per LNP sample, sized and labeled appropriately for the following: - RNA solution: a tube for the RNA aqueous solution (1 to 15 mL) - Concentrated sample: a collection tube for the RNA-LNP sample, prior to 0.2 µm filtration (1 to 15 mL) - RiboGreen sample: a collection tube for a small aliquot of the RNA-LNP for the RiboGreen assay (~20 to 25 µL) - Final sample: a collection tube for the RNA-LNP sample, for the remaining RNA-LNP for cell culture after the 0.2 µm filtration (1 to 15 mL) • 200 µL and 1 mL micropipettes and sterile pipette tips • MWCO centrifugal filters • Syringes, needles and 0.2 µm filters

6	Prepare the 1X dilution buffer in a tube according to the volumes of 10X dilution buffer (1) and molecular grade water (2) listed in Step 2 in the *Workbook*.

Step 2. Dilution Buffer Preparation	
Dilution buffer (10X) (mL)	3 (1)
Molecular grade water (mL)	27 (2)
Final volume (mL)	30.0

7	In the BSC, prepare the MWCO filters as follows: **a.** Rinse the filters with 70% isopropanol.

Step	Action
	b. Spin approximately 15 mL of ultra-pure molecular grade water through the filter at 4000 x g for 5 minutes. ***Note:*** *The water spin removes membrane preservatives and allows you to verify the membrane integrity. If water passes through the filter fully, the membrane might be compromised, and the filter should be discarded.*

Prepare RNA solution

Introduction

This section describes the procedure to prepare the RNA solution. There are two different procedures, depending on the type of RNA. All steps must be performed inside a BSC to minimize bacterial contamination and potential growth within the solutions.

Before starting, measure the concentration of the RNA stock solution(s) with UV-Vis to ensure the concentration is as expected.

Prepare mRNA for one-component delivery

Step	Action
1	Pipette the formulation buffer (1) and molecular grade water (2) volumes indicated in Step 3 of the *Workbook* into a tube for the RNA solution.
2	Mix well.
3	Pipette the mRNA volume (3) indicated in Step 3 of the *Workbook* into the RNA solution tube.

Step 3. RNA Solution Preparation	
Molecular grade water (mL)	516.6 (1)
Formulation buffer (µL)	88.0 (2)
mRNA (µL)	275.4 (3)
Final volume (µL)	880.0

Keep the RNA stock solution on ice until use.

Prepare mRNA for two-component delivery

Step	Action
1	Pipette the formulation buffer (1) and molecular grade water (2) volumes indicated in Step 3 of the *Workbook* into a tube for the RNA solution.

Step 3. RNA Solution Preparation		
Molecular grade water (mL)	516.6	1
Formulation buffer (µL)	88.0	2
Cas9 mRNA (µL)	137.7	3
sgRNA (µL)	137.7	4
Final volume (µL)	880.0	

2	Mix well.
3	Pipette the Cas9 mRNA (3) and sgRNA (4) volumes indicated in Step 3 of the *Workbook* into the RNA solution tube.

Keep the RNA stock solution on ice until use.

Formulate RNA-LNPs

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

Step	Action
1	Turn on the instrument as described in the instrument *Operating Instructions*.
2	On the main menu, tap ***Quick Run***.
3	Input the calculated parameters as given in Step 5 of the *Workbook*. Refer to the instrument *Operating Instructions* for details.

Step 5. Ignite Parameters	
C (aq) syringe **volume** (mL)	1
R (lipid) syringe **volume** (mL)	1
Flow rate ratio C:R	2:1
Total volume (mL)	1.1
Total flow rate (mL/min)	12
Start waste (mL)	0.10
End waste (mL)	0

4	Insert the NxGen cartridge into the instrument.
5	Prepare the **R** syringe with the lipid mix:

Step	Action
	a. Choose the syringe size with the volume specified for the **R** syringe in Step 5 of the *Workbook*. **b.** Draw the required amount of lipid mix into the syringe using a clean blunt needle. **c.** Remove the needle from the syringe, and tap the syringe to clear any air bubbles. **d.** Use the plunger to advance the liquid in the syringe, making sure to avoid drips from the tip of the syringe.
6	Prepare the **C** syringe with the aqueous solution: **a.** Choose the syringe size with the volume specified for the **C** syringe in Step 5 of the *Workbook*. **b.** Draw the entire solution from the RNA solution tube into the syringe using a clean blunt needle. **c.** Make sure the appropriate volume is present in the **C** syringe. **d.** With the needle in place, tap the syringe to clear any air bubbles. **e.** Use the plunger to advance the liquid in the syringe, making sure to avoid drips from the tip of the syringe.
7	Continue to attach the syringes 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 to downstream process the LNPs and optionally prepare the final sample for long term storage at -80°C.

Step	Action
1	Perform a 30X dilution of the RNA-LNPs with 1X dilution buffer in the BSC.
2	Fill the MWCO centrifugal filter or filters with the diluted RNA-LNPs, as calculated in step 6.

Step 6. Downstreaming Processing	
Number of centrifugal filters	1
Target concentrated vol. (mL)	1.0
RNA theoretical max (µg)	229

3	Spin at 4000 x g for 10 minutes at 4°C in a swing bucket rotor. ***Note:*** *Lower speeds are acceptable but increase the processing time.*

Step	Action
4	Discard the solution below the filter unit and repeat *step 3* as necessary until the entire sample is re-concentrated to approximately the starting RNA-LNP volume.
5	In the BSC, recover the sample from the filter using a micropipette.
6	Wash the MWCO centrifugal filter membrane with approximately 100 to 200 µL 1X dilution buffer to increase the recovery of RNA-LNPs.
7	Pipette the sample into an RNase-free tube.
8	Optional. Prepare the RNA-LNPs for long-term storage: **a.** Dilute aliquots of the LNPs 1:1 with the cryopreservation buffer included in the kit. **b.** Mix the LNPs with the buffer thoroughly by pipetting up and down at least five times.
9	In the BSC, filter the concentrated sample using a sterile 0.2 µm filter into the tube for the final sample. ***Tip:*** *To maximize LNP recovery:* *a. Wet the filter with small amounts of 1X dilution buffer (kept in a separate syringe).* *b. Filter the LNP sample.* *c. Further filter 100 to 200 µL 1X dilution buffer after the LNP sample has been collected.* *d. Finally, push air through the filter two to three times to make sure all residual LNPs are collected.* ***Note:*** *The use of multiple 0.2 µm filters might be needed for larger sample volumes. Use of multiple filters is not expected to impact RNA-LNP yield.*
10	Aliquot 25 µL of the RNA-LNPs into the tube for the RiboGreen sample.
11	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 *Access user documentation online, on page 3*.
12	Optional: Measure the size of the particles by dynamic light scattering (DLS).

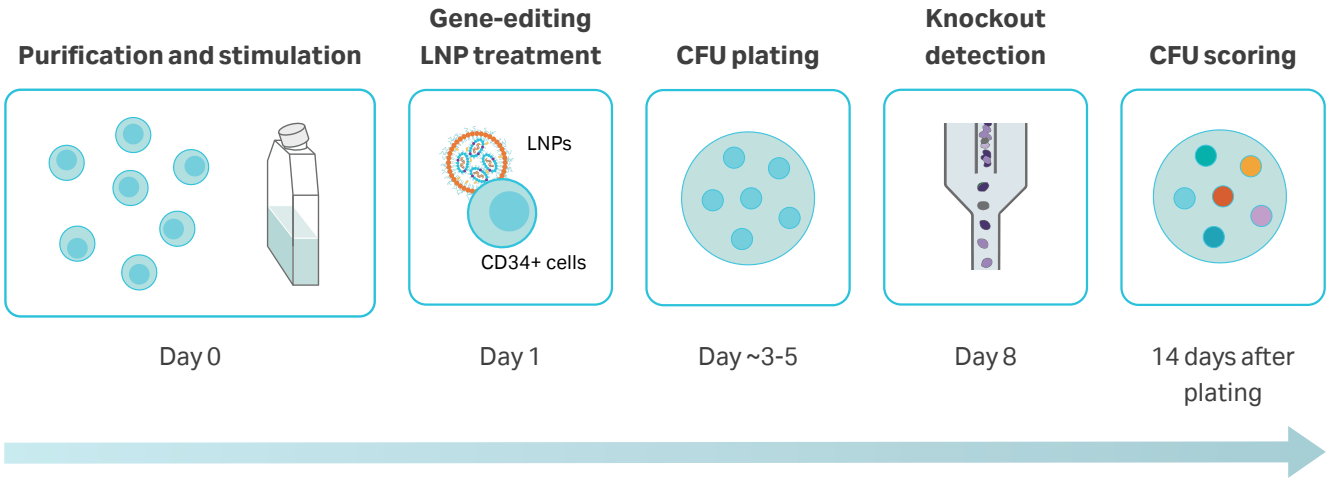
The RNA-LNPs are now ready for use. For short-term storage, store the sample at 4°C and use it for cell treatment within 1 week. For long-term storage, store formulated RNA-LNPs in the cryopreservation buffer at -80°C for up to one month.

Note: *Storage for longer than one month requires testing.*

LNP treatment of HSCs

Overview

The illustration below shows a schematic diagram of the HSC cell culture and LNP treatment work-flow. The suggested time points in the workflow are based on model experiments knocking out CD45 and CD33. Refer to *Genome Editing of CD34+ Hematopoietic Stem and Progenitor Cells with Lipid Nanoparticles* for more details.



Cell culture recommendations

For optimal LNP transfection efficiency, consider the following critical parameters:

- **RNA-LNP dosage:** It is recommended to perform a complete dose titration for each payload to find the optimal dose. A payload can be, for example, a different construct of single guide RNA. The recommended range for the titration dose can be from 0.2 to 20 µg total RNA per million cells treated.
- **Sterile practice:** Maintain a sterile work environment when handling cells and LNPs. Operators should be trained on proper sterile technique prior to working inside the biosafety cabinet.
- **Cell thawing and expansion:** Cryopreserved CD34+ HSCs should be stimulated for 24 hours after thawing, prior to LNP addition.
- **Cell density:** Seeding density between 0.1 to 0.5 million cells/mL is recommended for LNP treatment.
- **LNP treatment time:** Incubation time post LNP transfection depends on the characteristics of the RNA payload, for example, expression kinetics and stability. A 24 to 96 hour treatment is recommended, with further optimization as required.
- **Cell culture media:**: The Cytiva CD34+ HSC LNP kit shows optimal performance in serum-free media. A cell culture medium with low serum (≤ 1%) at the time of LNP addition might be acceptable, but must be experimentally validated. Further, using a serum-free medium is recommended for optimal retention of stemness and engraftable phenotype (CD34+ CD38- CD90+ CD133+).

LNP treatment procedure

The steps below give an overview of the LNP treatment procedure. Refer to the *Genome Editing of CD34+ Hematopoietic Stem and Progenitor Cells with Lipid Nanoparticles* for a detailed example workflow that has been optimized for LNP treatment of HSCs, and troubleshooting tips. See *Related documentation, on page 2*.

Step	Action
1	Thaw or isolate CD34+ HSCs.
2	Dilute cells to 0.1 to 0.5 million cells/mL in cell culture medium.
3	The following day, just prior to LNP treatment, add ApoE to the cells as follows: **a.** Prepare 0.1 mg/mL ApoE stock solution by diluting the included 500 µg ApoE with 5 mL 1X PBS without calcium or magnesium. Store at -80°C for up to 2 months in aliquots to avoid freeze thaws. **b.** Add the ApoE stock solution to the cells to achieve final concentration of 1 µg/mL (1:100 dilution of 0.1 mg/mL stock). **c.** Mix thoroughly by trituration.
4	Seed the cells into culture vessel or well plate.
5	Add formulated LNPs directly into the seeded cells.
6	Incubate at 37°C, 5% CO_2.
7	Perform downstream analysis to assess transfection efficiency, such as flow cytometry, or a colony-forming unit assay.

Ordering information

Visit precisionnanosystems.com to find the latest information.

Name	Product code
Cytiva CD34+ HSC LNP kit, 2 mL	1005000
NanoAssemblr Ignite instrument	NIN0001
NanoAssemblr Ignite+ instrument	1001413
Cytiva CD34+ HSC LNP kit, 100 µL	1003000
Cytiva CD34+ HSC LNP kit, 100 µL with cartridges	1004000
NanoAssemblr NxGen Ignite cartridges - 100 pack	NIN0061
NanoAssemblr NxGen Ignite cartridges - 200 pack	NIN0062

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