Cytiva™ CD34+ HSC LNP kit, 100 µL

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, 100 µL.

Before using this product, all users must read this document and the *NanoAssemblr™ Spark™ Operating Instructions (NIS1024)*.

Intended use

Cytiva CD34+ HSC LNP kit, 100 µL 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 Spark instrument and Spark 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, 100 µL is a lipid nanoparticle (LNP) reagent mix optimized for the delivery of RNA (such as Cas9 mRNA/sgRNA) into stimulated HSCs.

The Cytiva CD34+ HSC LNP kit components are used with the NanoAssemblr Spark instrument to produce RNA-LNPs. The kit is available in two configurations. Both configurations include reagents, while only one includes Spark cartridges. See *Kit components, on page 4*.

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 for *ex vivo* gene delivery and editing. The kit is suitable for use in drug discovery, screening, and 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 preclinical scale applications with the NanoAssemblr Ignite™ or Ignite+™ instrument, the following products can be used.

- Cytiva CD34+ HSC LNP kit, 2 mL
- NanoAssemblr Ignite instrument
- NanoAssemblr Ignite+ instrument

See *Find ordering information online, on page 18* for more information.

Related user resources online

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

- *Cytiva CD34+ HSC LNP kit, 100 µL Workbook*
- *RiboGreen Assay Protocol to Determine RNA Encapsulation Efficiency*
- *Genome Editing of CD34+ Hematopoietic Stem and Progenitor Cells with Lipid Nanoparticles*

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 Spark Operating Instructions (NIS1024) This document is referred to as *the instrument Operating Instructions* in this document.	Instructions needed to prepare and operate the Spark 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

Introduction

The kit is available in two configurations, one that includes Spark cartridges, and one that does not. The following sections give the component names and storage temperatures for each configuration of the kit.

Cytiva CD34+ HSC LNP kit (1003000)

Label	Content	Size	Storage
Lipid mix	Lipid mix	100 µL	-80°C
Formulation buffer type 1	Formulation buffer	400 µL	2°C to 8°C
Dilution buffer type 1	Dilution buffer	1500 µL	2°C to 8°C
Apolipoprotein-E3 (ApoE3)	Apolipoprotein-E3 (ApoE3)	100 µg	-80°C

Cytiva CD34+ HSC LNP kit with cartridges (1004000)

Label	Content	Size	Storage
Lipid mix	Lipid mix	100 µL	-80°C
Formulation buffer type 1	Formulation buffer	400 µL	2°C to 8°C
Dilution buffer type 1	Dilution buffer	1500 µL	2°C to 8°C
Apolipoprotein-E3 (ApoE3)	Apolipoprotein-E3 (ApoE3)	100 µg	-80°C
NanoAssemblr Spark cartridges		5 pieces	15°C to 25°C

Expiry

See individual component packaging.

Kit capacity

The kit contains sufficient materials for preparation of at least 5 lipid nanoparticle formulations, each containing 10 µg of RNA.

Required materials

Required materials supplied by Cytiva

- NanoAssemblr Spark instrument
- NanoAssemblr Spark cartridges

See *Find ordering information online, on page 18* for more information.

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
- 0.5 to 2 mL tubes with screw cap and O-ring seal
- Micropipettes and RNase free pipette tips – 10, 20, and 200 µL
- 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 Spark 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.

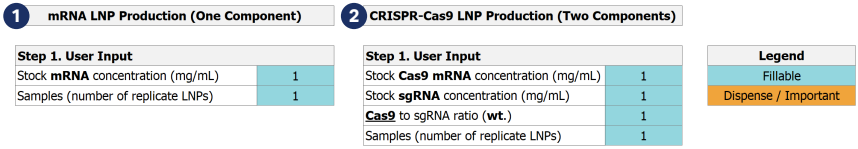
Phase	Action	Description
1	Fill in the *Cytiva CD34+ HSC LNP kit, 100 µL Workbook*	Calculate the reagent consumption and material preparation
2	Prepare the workspace and instrument	Thaw reagents and materials, label vials, and set up the Spark instrument
3	Prepare RNA solution	Prepare the RNA working solution by combining the RNA payload and the included formulation buffer
4	Formulate the RNA-LNPs	Formulate the LNPs on the Spark instrument
5	Quantify loaded RNA	Quantify the encapsulated RNA using the RiboGreen Assay
6	LNP treatment of HSCs	Add the RNA-LNPs to the cells

Fill in the calculation workbook

The *Cytiva CD34+ HSC LNP kit, 100 µL Workbook* is a tool to calculate parameters and volumes for the LNP formulation on the Spark instrument.

The *Cytiva CD34+ HSC LNP kit, 100 µL Workbook* is also referred to as the *Workbook* in this document. The *Workbook* is available on the web. See *Related user resources online, on page 2*.

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 mRNA LNP Production (One Component)	
Step 1. User Input	
Stock **mRNA** concentration (mg/mL)	1
Samples (number of replicate LNPs)	1

2 CRISPR-Cas9 LNP Production (Two Components)	
Step 1. User Input	
Stock **Cas9 mRNA** concentration (mg/mL)	1
Stock **sgRNA** concentration (mg/mL)	1
Cas9 to sgRNA ratio (**wt.**)	1
Samples (number of replicate LNPs)	1

Legend
Fillable
Dispense / Important

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
`Stock mRNA concentration (mg/mL)` [1]	Concentration of mRNA stock solution	User determined
`Stock Cas9 mRNA concentration (mg/mL)` [2]	Concentration of Cas9 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 (X:1)	1

Parameter	Description	Recommended value
Samples (number of replicate LNPs)	The number of LNP sample replicates	User determined

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

Prepare the workspace and the instrument

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 Spark instrument.

Step	Action
1	Turn on and clean the biosafety cabinet (BSC) by wiping it down with 70% isopropanol and RNase decontamination solution.
2	Set up the Spark instrument within the BSC. For detailed instructions on installation and operation, refer to the instrument *Operating Instructions*. **a.** Wipe down the instrument with 70% isopropanol. **b.** Connect the instrument to a grounded power outlet. **c.** Turn on the instrument.
3	Retrieve the frozen RNA aliquot(s) and thaw on ice. Always keep the RNA on ice to prevent material degradation.
4	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. Keep the vial closed to prevent evaporation.
5	Vortex the lipid mix tube to ensure homogeneity. Spin down in a microcentrifuge for 3 to 5 seconds.

Step	Action
6	Place the following items in the BSC:

On ice:	At room temperature:
• RNA aliquot(s)	• Spark instrument and cartridges • The kit components: lipid mix, formulation buffer, and dilution buffer • Three 0.5 mL tubes per LNP sample, labeled appropriately for the following: - RNA solution: a tube for preparing RNA aqueous solution - 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 (~150 µL) • 20 µL and 200 µL micropipettes and sterile pipette tips

7	On the instrument menu, tap ***Purge***, then press the physical button on the Spark instrument. *Result:* The instrument is purged to remove trapped air.
8	Tap ***Formulation*** on the user interface.
9	When prompted, set the instrument to pressure setting ***3***.

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.

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

Prepare mRNA for one-component delivery

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

Step 2. RNA Solution Preparation		
Molecular grade water (µL)	20.66	1
Formulation buffer (µL)	3.52	2
mRNA (µL)	11.02	3
Dispensed volume to cartridge (µL)	32.00	

Keep the RNA stock solution on ice until use.

Prepare mRNA for two-component delivery

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

Step 2. RNA Solution Preparation		
Molecular grade water (µL)	20.66	1
Formulation buffer (µL)	3.52	2
Cas9 mRNA (µL)	5.51	3
sgRNA (µL)	5.51	4
Dispensed volume to cartridge (µL)	32.00	

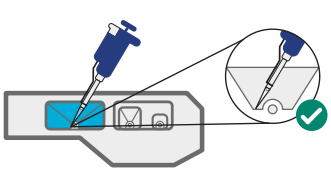
Step	Action
3	Pipette the Cas9 mRNA (3) and sgRNA (4) volumes indicated in Step 2 of the *Workbook* into the RNA solution tube.

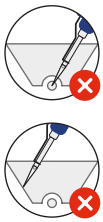
Keep the RNA stock solution on ice until use.

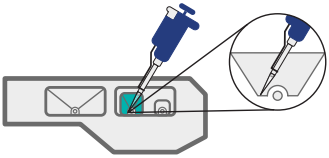
Formulate RNA-LNPs

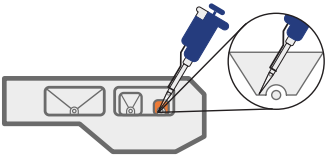
Follow the steps below to formulate the RNA-LNPs on the Spark instrument.

Step	Action
1	Inside the BSC, open the package containing the Spark cartridge and the accompanying cap.
2	Slowly fill well 1 of the Spark cartridge with dilution buffer using a micropipette. Make sure to position the pipette following the pipette positioning shown below to avoid bubble formation near the channel opening.
3	Fill well 2 of the cartridge with the RNA Solution prepared previously.
4	Fill well 3 of the cartridge with the lipid mix. Make sure to tightly cap the lipid mix vial immediately after use to decrease the risk of evaporation of this ethanol-based solution.
5	Cap the cartridge immediately to avoid evaporation of ethanol.
6	Insert the capped cartridge containing prepared reagents into the Spark instrument.









Step	Action
7	Set up and start the run as described in the instrument *Operating Instructions*.
8	Press the physical button on the Spark instrument to formulate the RNA-LNPs.

Collect formulated RNA-LNP sample

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

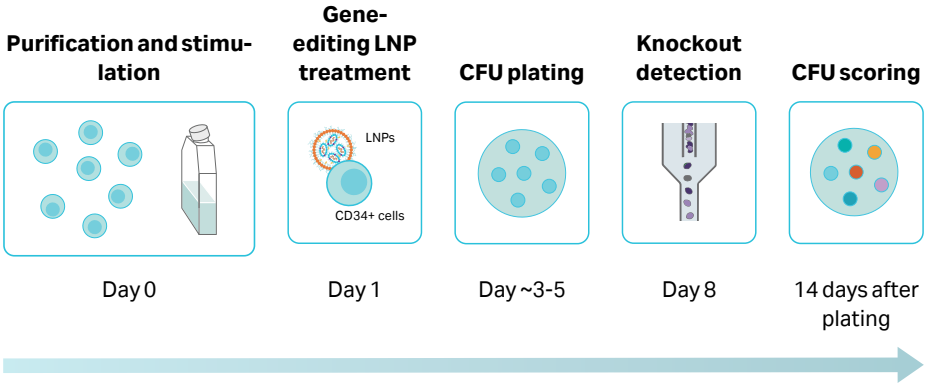
Step	Action
1	When instructed by the instrument, remove the Spark cartridge from the instrument.
2	Lift the cap off the cartridge carefully.
3	Using a micropipette, remove the RNA-LNP solution from well 1 of the cartridge, and add it to the sterile 0.5 mL microcentrifuge vial for the final sample.
4	Add 96 µL of dilution buffer to the formulated RNA-LNPs and pipette up and down to thoroughly mix. ***Note:*** *This 1:1 dilution step stabilizes the LNPs.*
5	Aliquot 25 µL of the RNA-LNPs into the tube for the RiboGreen sample.
6	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*.

The RNA-LNPs are now ready for use. Store the sample at 4°C and use it for cell treatment within 1 week.

LNP treatment of HSCs

Overview

The illustration below shows a schematic diagram of the HSC cell culture and LNP treatment workflow. 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 user 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 100 µg ApoE with 1 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.

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.

Page intentionally left blank

Give feedback on this document

Visit cytiva.com/techdocfeedback or scan the QR code.

cytiva.com

For local office contact information, visit cytiva.com/contact

1002515 AA V:7 06/2024