GenVoy-ILM™ T cell kit for mRNA, Ignite™

Instructions for Use

Introduction

Read this before unpacking or using the kit

This instruction contains information that is important for the safe handling, unpacking, and preparation of GenVoy-ILM™ T cell kit for mRNA, Ignite™, 3 mL and 6 mL.

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

Intended use

GenVoy-ILM T cell kit for mRNA, Ignite is a research use only kit for the delivery of RNA into activated human primary T cells.

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

GenVoy-ILM T cell kit for mRNA, Ignite is a lipid nanoparticle (LNP) reagent mix optimized for the delivery of RNA such as Cas9 mRNA/sgRNA into activated human primary T cells. The kit is available in two sizes: 3 mL and 6 mL.

The kit includes an ionizable lipid mix and all the required reagents to prepare LNPs on a NanoAssemblr Ignite or Ignite+ instrument.

This non-viral delivery method can be integrated into standard human T cell culture workflows using an established protocol with either freshly isolated or cryopreserved T cells. The kit enables researchers to establish a clinically relevant method for *ex vivo* gene delivery to accelerate the development of T cell therapies. The kit is suitable for preparations of mRNA-LNPs at the preclinical scale.

Typical applications

- Deliver mRNA to express proteins of interest, such as chimeric antigen receptors (CARs)
- Deliver nuclease mRNA for genetic engineering, such as gene knockouts

Related products

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

- GenVoy-ILM T cell kit for mRNA, Spark
- GenVoy-ILM T cell kit for mRNA, Spark with cartridges
- NanoAssemblr Spark instrument

See *Find ordering information online, on page 23* for more information.

Related user resources online

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

- *GenVoy-ILM T cell kit for mRNA, Ignite Workbook*
- *Genome editing of human primary T cells with lipid nanoparticles: Application note*
- *RiboGreen Assay Protocol to Determine RNA Encapsulation Efficiency*
- *RNA Quantification Workbook*

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.

General mRNA considerations

Introduction

The following section contains important considerations for preparing the mRNA to make sure the GenVoy-ILM T cell kit performs as expected. It is recommended that the RNA has the following characteristics to achieve maximum biological activity.

mRNA modifications

- 5′ capping and 3′ polyadenylation (polyA) protect against enzymatic degradation and are critical for biological activities.
- Experimental results show that Cap 1 modification results in significantly higher *in vitro* translation efficiency in T cells compared to Cap 0 (e.g., ARCA).
- Wild type (WT) bases have shown significantly higher expression levels in T cells *ex vivo* in experiments.

mRNA and guide RNA purity

- Use the highest purity mRNA available.
- When using in-house synthesized IVT RNA (both for mRNA and sgRNA), it is recommended to use gel electrophoresis to verify integrity. A single, sharp band of the expected size is expected.
- The encapsulation of impurities yields reduced performance because non-functional RNA displaces the positive lipid charges.
- All RNA stock solutions must have a concentration of at least 0.35 mg/mL for preparation of the RNA working solution.

Storage and handling

- The mRNA should be stored in a low ionic strength buffer, such as 1 mM sodium citrate at pH 6.4 or in RNAse-free water, as high levels of ions in solution may impact encapsulation efficiencies.
- For sgRNA, it is recommended to resuspend the pellet in molecular grade water.
- For all RNAs, avoid multiple freeze-thaw cycles by storing the solutions in aliquots at -80°C.

Kit components

Introduction

The kit is available in two sizes. The following sections give the component names and storage temperatures for each configuration of the kit.

GenVoy-ILM T cell kit for mRNA, Ignite, 3 mL (1001144)

Component	Size	Storage
Lipid mix	3 mL	-80°C
Formulation buffer (10X)	2 mL	2°C to 8°C
Dilution buffer (10X)	40 mL	2°C to 8°C
Cryopreservation buffer	12 mL	-80°C
Apolipoprotein-E3 (ApoE)	500 µg	-80°C

GenVoy-ILM T cell kit for mRNA, Ignite, 6 mL (1001161)

Component	Size	Storage
Lipid mix	6 mL	-80°C
Formulation buffer (10X)	4 mL	2°C to 8°C
Dilution buffer (10X)	80 mL	2°C to 8°C
Cryopreservation buffer	24 mL	-80°C
Apolipoprotein-E3 (ApoE)	500 µg × 2	-80°C

Expiry

See individual components.

Kit capacity

The kit contains sufficient materials for preparation of the following:

Number of batches[1]	LNP batch size	RNA input per sample[2]	T cell treatment batch (high dose)[3]	T cell treatment batch (low dose)[3]
GenVoy-ILM T cell kit for mRNA, Ignite, 3 mL				
1	8 mL	2 mg	100 million cells	1 billion cells
4	2 mL	0.5 mg	25 million cells	250 million cells
8	1 mL	0.3 mg	13 million cells	125 million cells
16	0.5 mL	0.13 mg	6 million cells	63 million cells
GenVoy-ILM T cell kit for mRNA, Ignite, 6 mL				
1	16 mL	4 mg	200 million cells	2 billion cells
4	4 mL	1 mg	50 million cells	500 million cells
8	2 mL	0.5 mg	25 million cells	250 million cells
32	0.5 mL	0.13 mg	6 million cells	63 million cells

[1] Number of batches assumes a minimum LNP production volume of 0.5 mL per run.
[2] Input RNA is calculated based on the instructions in this document.
[3] T cell estimations assume that there is 10 µg RNA per million T cells in a high dose treatment, and 1 µg RNA per million T cells in a low dose treatment.

An optimal treatment dose is expected to fall between 1 to 10 µg RNA per million cells.

Required materials

Required materials supplied by Cytiva

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

See *Find ordering information online, on page 23* for more information.

Required materials supplied by the user

To prepare RNA-LNPs with the GenVoy-ILM T cell 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
- Bead bath or heat block for thawing kit components
- 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.*
- Molecular grade water (RNase/DNase and endotoxin-free)
- 70% isopropanol or ethanol
- RNase decontamination solution
- 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 working solution preparation, instrument handling, and LNP preparation. A typical workflow using a GenVoy-ILM T cell kit is given in the table below.

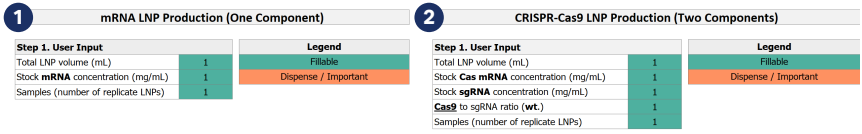
Phase	Action	Description
1	Fill in the *GenVoy-ILM T cell kit for mRNA, Ignite Workbook*	Calculate the reagent consumption and material preparation
2	Prepare the workspace, reagents, and 1X dilution buffer in a biosafety cabinet	Thaw reagents and materials, label tubes, and prepare 1X dilution buffer
3	Prepare RNA working 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 T cells	Add the RNA-LNPs to the cells

Fill in the calculation workbook

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

The *GenVoy-ILM T cell kit for mRNA, Ignite 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. 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

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

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). T 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.
4	Vortex the lipid mix tube to ensure homogeneity. Spin down in a centrifuge for 3 to 5 seconds.
5	Place the following items in the BSC: On ice: • RNA aliquot(s) At room temperature: • 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 working solution: a tube for the RNA working 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

Step	Action
	• 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. **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 the RNA working solution

Introduction

This section describes the procedure to prepare the RNA working 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 the mRNA for one-component delivery

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

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

Keep the RNA working solution on ice until use.

Prepare the mRNA for two-component delivery

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

Step 3. RNA Working Solution Preparation	
Molecular grade water (µL)	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

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

Keep the RNA working 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: **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.** Make sure the appropriate volume is present in the **R** syringe as indicated in Step 4 of the *Workbook*. **d.** With the needle in place, tap the syringe to clear any air bubbles. **e.** Remove the needle from the syringe. **f.** 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 RNA working solution (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 working solution tube into the syringe using a clean blunt needle. **c.** Make sure the appropriate volume is present in the **C** syringe.

Step	Action
	d. With the needle in place, tap the syringe to clear any air bubbles.
	e. Remove the needle from the syringe.
	f. 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 the 1X dilution buffer that was prepared earlier.
2	Fill the MWCO centrifugal filter or filters with the diluted RNA-LNPs, as calculated in Step 6.

Step 6. Downstream 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.*
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 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:*

Step	Action
	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 *Related user resources online, on page 2*.
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 T cells

Overview

The table below shows a timeline of the standard T cell culture and LNP treatment workflow.

Refer to *Genome Editing of Human Primary T cell with Lipid Nanoparticles* for more details. See *Related user resources online, on page 2*.

Workflow phase	Therapeutic protein expression	Gene knockouts
Primary T cell isolation or thaw	Day 0	
Cell activation and expansion	Day 0–3	User determined, based on treatment day
RNA-LNP preparation: • RNA loaded LNP production • LNP purification and RNA quantification	Day 0–3	User determined, based on treatment day
RNA-LNPs treatment	Day 3 (CAR mRNA-LNPs)	Day 1–3 (CRISPR RNA-LNPs)
Gene editing onset and cell expansion	N/A	Days 4–8
Flow cytometry or functional assays	Day 4–5	Day 7–9

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 (0.2 to 20 µg total RNA per million cells) for each payload to find the optimal dose. A payload can be, for example, a different construct of sgRNA.

  Note: *An optimal treatment dose is expected fall between 1 to 10 µg RNA per million cells.*

- **Sterile practice:** Maintain a sterile work environment when handling cells and LNPs.

- **Cell thaw, activation, and expansion:** Primary T cells require activation to express mRNA at sufficient levels. For protein expression, a 72 hour activation prior to LNP treatment is recommended. For Cas9 knockouts, the activation time can be reduced, as Cas9 expression levels can be lower. In the absence of an experiment to determine the optimal activation time, 72 hours is generally recommended as the activation time for all applications.
- **Cell density:** Seeding density between 0.25 to 0.5 million cells/mL is recommended for LNP treatment.

  Note: *Lower seeding density is better for optimal protein expression.*
- **Incubation time post LNP transfection:** The incubation time depends on the characteristics of the RNA payload, for example, expression kinetics and stability. A 24 to 96 hour incubation is recommended, with further optimization as required.
- **Cell expansion post LNP treatment:** A wash step or media exchange is recommended 24 hours post-LNP treatment to replenish nutrients. This step supports optimal protein expression and enhances downstream detection with flow cytometry.
- **Cell culture media:** The GenVoy-ILM T cell kit shows optimal performance in commercially available serum-free media, as described in *Genome Editing of Human Primary T cell with Lipid Nanoparticles*. Addition of any additional serum during the LNP treatment is detrimental to the LNP transfection.

LNP treatment procedure

The steps below give an overview of the LNP treatment procedure. Refer to *Genome Editing of Human Primary T Cells with Lipid Nanoparticles Gene-edited CAR T cells for next-generation cell therapies* for a detailed example workflow that has been optimized for LNP treatment of T cells, and troubleshooting tips. See *Related user resources online, on page 2*.

Step	Action
1	Immediately prior to LNP treatment, prepare the cells as follows: **a.** Gently pipette the cell to obtain single cell suspension. **b.** Count the cells.
2	Dilute cells to the required seeding density in cell culture media containing 100 ng/mL Interleukin-2 (IL-2). Supplement the diluted cell suspension with ApoE to a final concentration of 1 µg/mL as follows:

Step	Action
	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. ***Note:*** *ApoE stock solution can be stored at -80°C for up to 2 months. Store in aliquots to avoid freeze-thaw cycles.* **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.
3	Seed the diluted cells into a culture vessel or a well plate.
4	Add the formulated LNPs directly into the seeded cells with a sterile pipette tip, and gently mix the cell suspension with the same tip.
5	Incubate at 37°C, 5% CO_2.
6	24 hours after the LNP treatment: **a.** Centrifuge the plate at 300 x g for 10 minutes at room temperature. **b.** Discard the supernatant. **c.** Top-up or resuspend the pellet in 250 µL of complete T cell culture media. **d.** Transfer to an incubator and continue the expansion of the cells.
7	Perform downstream analysis to assess transfection efficiency, such as flow cytometry, or a functional 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.

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