Application Note

mRNA Delivery to Activated Primary Human T Cells Using C14-4 Lipid Nanoparticles

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Key Features

- Primary immune cells, especially T cells, are challenging to transfect due to their natural resistance to foreign genetic material.
- Lipid nanoparticles (LNPs) offer improved efficiency with reduced cytotoxicity compared with traditional transfection methods.
- This workflow can be used with LNPs for the delivery of mRNA to achieve optimal protein expression in activated primary human T cells.

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Introduction

Primary immune cells, especially T cells, are challenging to transfect due to their natural resistance to foreign genetic material. Furthermore, these cell populations are sensitive to traditional transfection methods that have unwanted cytotoxicity, resulting in high rates of cell death.

Lipid nanoparticles (LNPs) offer improved transfection efficiency and reduced cytotoxicity. Recent studies show LNPs containing the ionizable lipid C14-4 can induce CAR expression in T cells as effectively as electroporation but with reduced cytotoxicity. However, optimal cell culture conditions for protein expression in T cells still need investigation. Herein, we report a workflow for mRNA delivery to activated primary human T cells.

Experimental Design

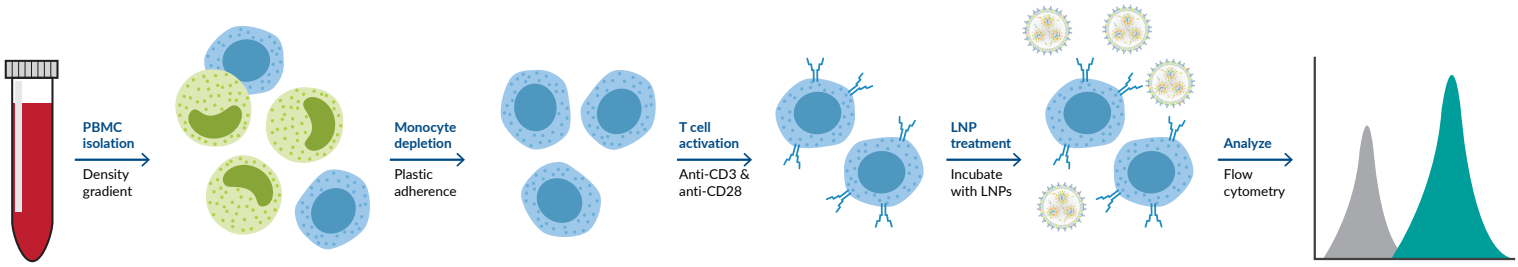

Figure 1. Schematic workflow of primary human T cell transfection with LNPs.

LNP Preparation

1. LNPs were prepared using Cayman's LipidLaunch™ C14-4 T Cell Exploration Kit.
2. The organic phase consisted of a lipid mix in ethanol (35:16:46.5:2.5 molar ratio of C14-4, 1,2-DOPE, cholesterol, and DMG-PEG(2000), respectively). The aqueous phase contained 200 µg/ml eGFP-mRNA in 50 mM sodium acetate, pH 4.5.
3. LNPs were formed by microfluidic mixing. The total flow rate was 10 ml/min with a flow rate ratio of 3:1 (mRNA:lipid).
4. LNPs were dialyzed against PBS, pH 7.4, overnight and stored at 4°C until use.

Biophysical Characteristics

1. LNP size and polydispersity was determined by dynamic light scattering (DLS).
2. mRNA concentration and encapsulation efficiency were determined using a fluorescent RNA dye in the absence and presence of 0.5% Triton X-100.
3. For all experiments, LNPs were diluted to the working mRNA concentration in complete media. For control (no LNP) conditions, eGFP-mRNA was diluted in PBS and added to media at equivalent concentrations.

Jurkat Cell Transfection with LNPs

1. Jurkat cells were seeded for LNP treatment at 100,000 cells per well (in 96-well plates).
2. C14-4 mRNA LNPs were added to Jurkat cells in media to a final eGFP-mRNA concentration of 125, 250, or 500 ng/ml.
3. Following treatment of the cells with LNPs for 24, 48, or 72 hours, cells were stained with 1 µg/ml DAPI and analyzed by flow cytometry.

Primary Human T Cell Transfection with LNPs

1. PBMCs were enriched from fresh whole blood using density centrifugation.
2. Monocytes were depleted by adherence to plastic.
3. T cells were activated in complete RPMI with plate-bound anti-CD3 (1 µg/ml) and soluble anti-CD28 (5 µg/ml) for three, four, five, or six days.
4. eGFP LNPs were added to primary human T cells in media to final mRNA concentrations of 125, 250, or 500 ng/ml and incubated for up to 72 hours.
5. Following treatment of the cells with LNPs, cells were stained with anti-CD3 antibody and DAPI and analyzed by flow cytometry.

Results & Discussion

LNP Analysis

LNPs prepared using the LipidLaunch™ C14-4 T Cell Exploration Kit had an average diameter of less than 100 nm and a low polydispersity index (PDI), indicating a narrow particle size distribution (**Figure 2**). C14-4 LNPs had good mRNA encapsulation efficiency, indicating minimal RNA loss during preparation.

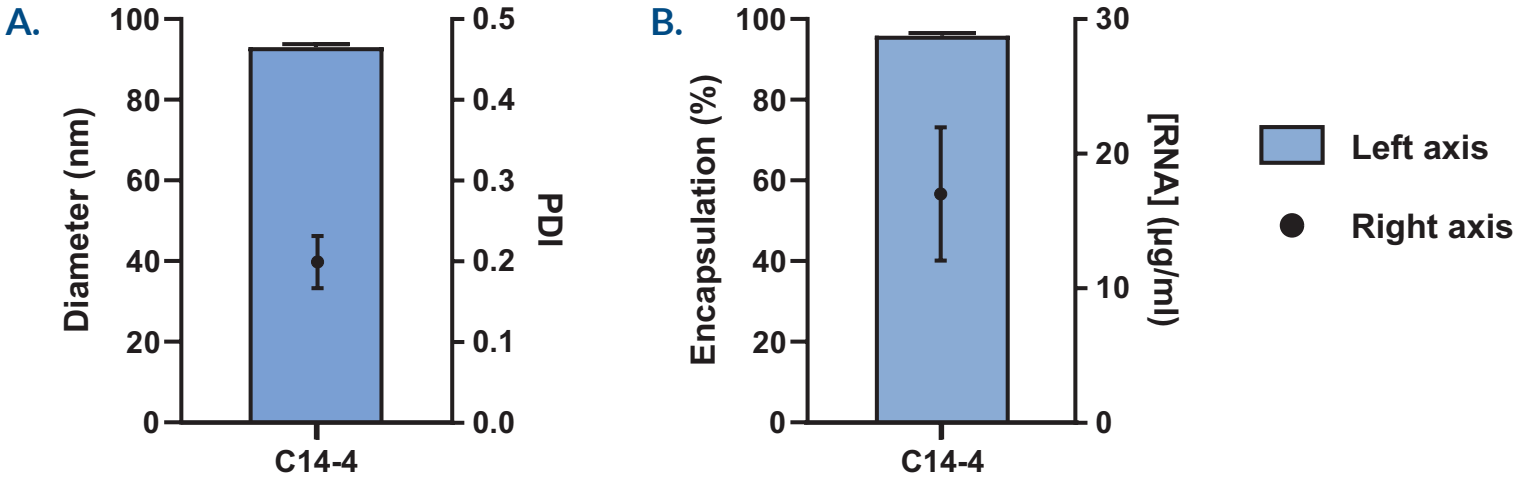

Figure 2. LNP particle characteristics. **A)** LNP average particle size and PDI were measured using dynamic light scattering (DLS). **B)** RNA encapsulation efficiency and concentration were quantified *via* a fluorescent RNA dye in the absence and presence of 0.5% Triton X-100.

Jurkat Cell Transfection with LNPs

Jurkat cells, an immortalized T cell line derived from the peripheral blood of a patient with T cell leukemia, represent a suitable model for initial testing of T cell transfection using LNPs. A time and dose experiment was performed with Jurkat cells to establish an appropriate protocol for transfection of activated T cells and assessment of transfection efficacy and viability. Maximal transfection efficiency, as measured both by intensity of eGFP and percentage of total cells expressing eGFP, was observed at about 48 hours after transfection, though it was detectable by 24 hours and remained high at 72 hours (**Figure 3**). Additionally, all LNP doses tested resulted in high expression of eGFP and viability, although by 72 hours after transfection, the highest dose did result in a small decrease in cell viability.

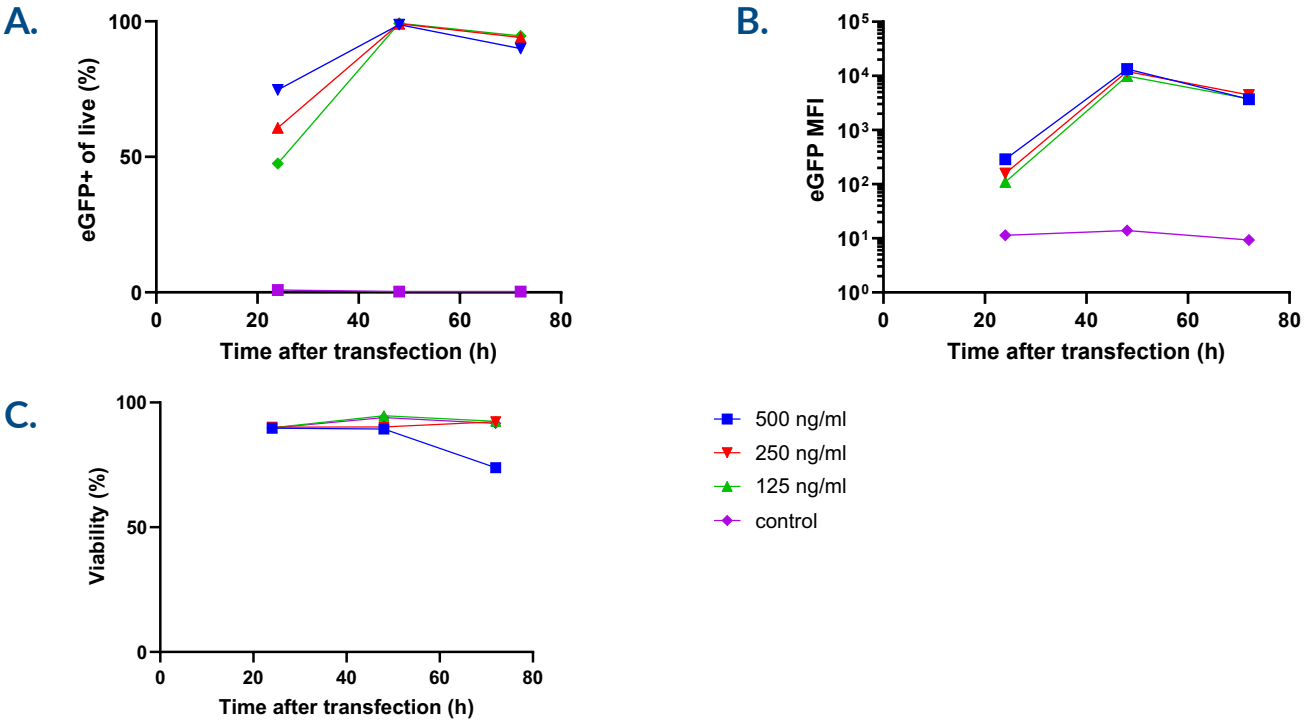

Figure 3. eGFP expression in Jurkat T cells after treatment with eGFP-mRNA LNPs. **A)** positivity rate for eGFP expression, **B)** eGFP protein expression levels (reported as mean fluorescence intensity), and **C)** viability of transfected and control cells.

Experiments with Primary T Cells

Next, we determined the optimal experimental parameters required for activation of primary human T cells prior to LNP treatment to achieve optimal protein expression.

Primary T Cell Activation

Primary T cells were activated with anti-CD3 (plate-bound) and anti-CD28 (soluble) for three, four, five, or six days. These timepoints were selected to provide sufficient time for the initiation of proliferation in the activated T cells. After activation, primary human T cells were incubated with 250 ng/ml eGFP-mRNA LNPs for 24 to 72 hours prior to analysis.

Optimal expression was observed in primary T cells with three day activation and 48-hour treatment with eGFP-mRNA encapsulated by C14-4 LNPs.

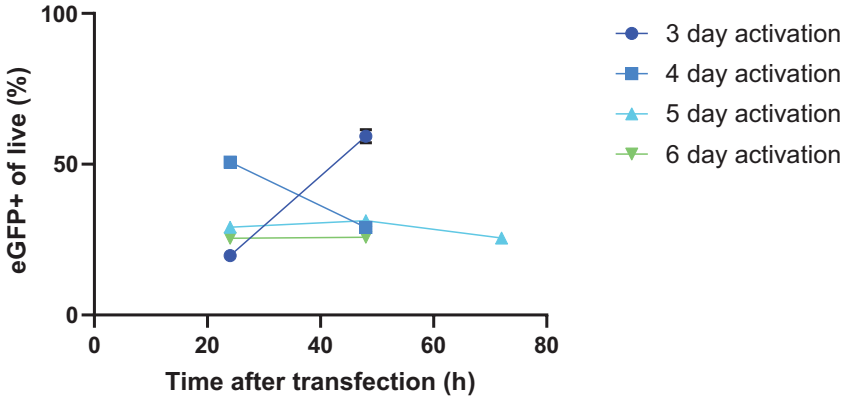

Figure 4. Kinetics of eGFP expression over 72 hours of LNP treatment after activation for the indicated time periods.

eGFP Dose-Response in Primary Human T Cells

We carried out a dose-response experiment to determine the impact of increasing concentrations of eGFP-mRNA on protein expression and cell viability in primary human T cells after three days of activation followed by 48 hours of treatment with LNPs.

eGFP expression increased in a dose-dependent manner using this workflow with C14-4 LNPs.

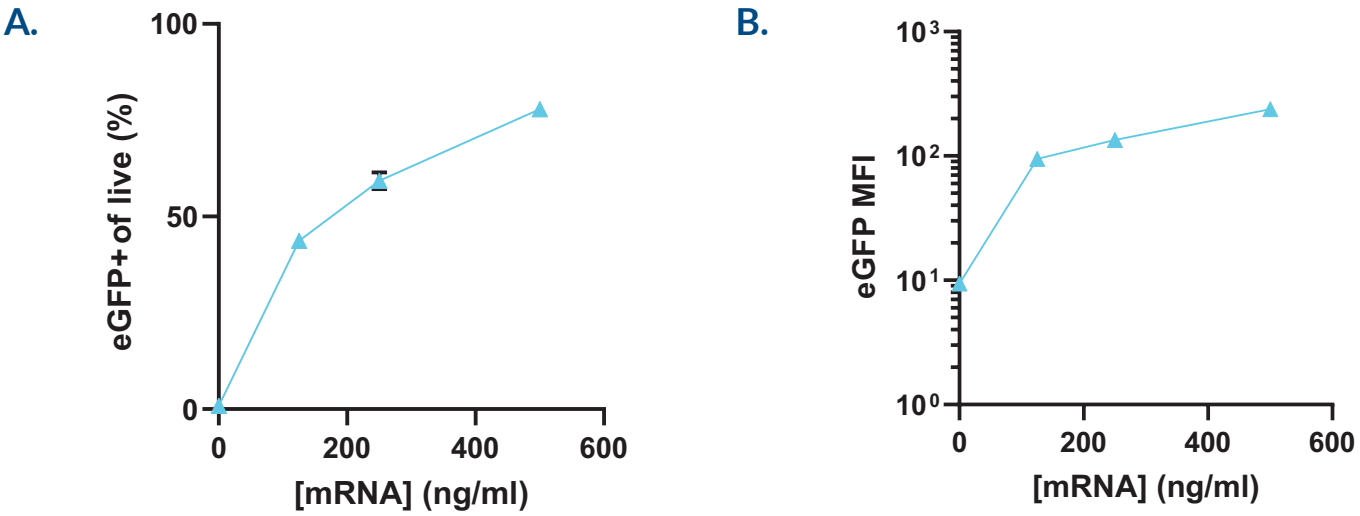

Figure 5. eGFP expression in primary human T cells after three days of activation and 48 hours of treatment with C14-4 eGFP-mRNA LNPs. **A)** percent of live cells positive for eGFP expression and **B)** eGFP protein expression levels (reported as mean fluorescence intensity).

Cell viability was maintained at high levels across the range of eGFP-mRNA doses tested using this workflow with C14-4 LNPs.

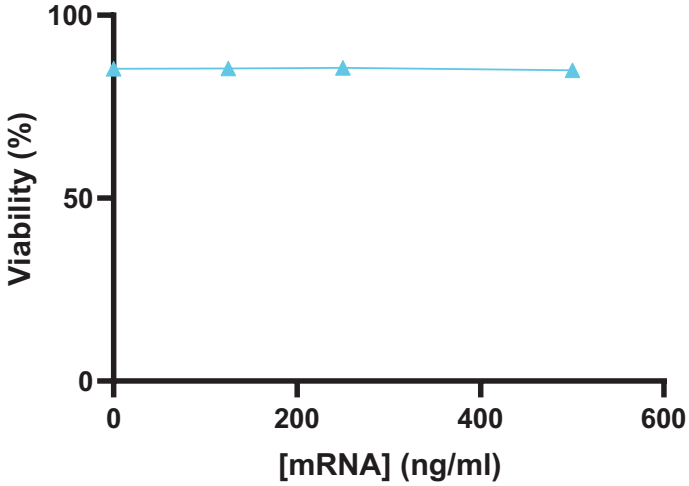

Figure 6. Viability analysis of primary human T cells after three days of activation and 48 hours of treatment with eGFP-mRNA LNPs.

Taken together, these data demonstrate that this workflow is an effective and non-toxic delivery method for mRNA in primary human T cells.

Appendix

Materials

LipidLaunch™ C14-4 T Cell Exploration Kit	Cayman Chemical Item No. 41820
mRNA	eGFP-mRNA in 50 mM sodium acetate, pH 4.5
RNA quantification	Fluorescent RNA dye ± 0.5% Triton X-100
Immortalized T cells	Jurkat cells
Primary T cells	Human peripheral blood T cells
Primary T cell activator	anti-CD3 (1 µg/ml, plate-bound) and anti-CD28 (5 µg/ml, soluble)
Cell media	RPMI with 10% FBS and 1% penicillin/streptomycin
Flow cytometry antibodies/stains	APC anti-human CD3 DAPI Cayman Chemical Item No. 40796

Equipment

LNP preparation	Sunshine microfluidic mixer – Unchained Labs
Particle analysis	Stunner – Unchained Labs
Flow cytometer	MACSQuant Analyzer – Miltenyi Biotec

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