

## Lipid Nanoparticle (SV104-Delivery™) R&D Kit

For human CD4 T cell specific delivery of mRNA

Catalog number: SV104-G

Table 1 Product contents

| Cat. No.                                           | Vol. or Wt. | Storage | Required materials not supplied   |
|----------------------------------------------------|-------------|---------|-----------------------------------|
| SVD-1                                              | 3 x 0.3 mL  | -80 °C  | •RNase-free water                 |
| SVD-2                                              | 1 x 1.5 mL  | R.T.    | •RNase-free PBS                   |
| SVD-3                                              | 1 x 3 mg    | -80 °C  | •Micropipettes                    |
| SVD-4-B4                                           | 1 x 0.4 mL  | 2-8 °C  | •RNase-free filter pipette        |
| Materials supplied for encapsulating 36 µg of mRNA |             |         | •RNase-free microcentrifuge tubes |
|                                                    |             |         | •Purification tube                |
|                                                    |             |         | •Cell Culture media               |
|                                                    |             |         | •Cytokines                        |

### Preparations of Reagents before use (!)

1. Lipid Mixture (SVD-1): This lipid mixture stock solution should be stored at -80 °C for long-term use.
2. Lipid mixture (SVD-1), formulation buffer (SVD-2), and reagent A (SVD-3): Allow these reagents to reach room temperature prior to use.
3. Reagent A (SVD-3): Add 1 mL of RNase free water to make a 10X stock solution (3 mg/mL). Aliquot 200 µL for each and store at -80 °C. Avoid freeze-thaw cycle. Dilute 10-fold with water to 1X before use.
4. **Reagent B4** (SVD-4-B4): **Do not freeze!** Avoid freeze-thaw cycle.

**Note:** For preparation of human PBMCs, please follow the manufacturer's protocol for the PBMC isolation kit (Stemcell Technologies or the instructions of other similar PBMC isolations kits).

## Warranty and limitation

The buyer agrees to purchase the kit containing materials to SunVax mRNA Therapeutics' Terms and Conditions.

Prior to use, the Buyer is responsible for requesting and reviewing the Complete Terms and Conditions including details of Warranty and Limitation of Liability, via SunVax mRNA Therapeutics website.

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**If you need large volumes of the cell type specific LNP-mRNA, please contact**

**SunVax\_Customer@sunvaxmrna.com.**

## Protocol for Lipid Nanoparticle (SV104-Delivery™) R&D kit

1. In a 1.5 mL RNase-free microcentrifuge tube, dilute 4 µg of mRNA (e.g., eGFP mRNA) in 100 µL of formulation buffer (SVD-2, prewarmed to R.T.). Mix thoroughly by pipetting up and down at least 5 times. Briefly centrifuge if there are bubbles.
2. Transfer 100 µL of the lipid mixture (SVD-1, prewarmed to R.T.) into a 1.5 mL RNase-free microcentrifuge tube.
3. Add 100 µL mRNA solution (in formulation buffer) into the 100 µL lipid mixture under vortex (2000 RPM) mixing in 10 seconds and incubate at 37 °C for 5 minutes (See the video below).  
<https://www.youtube.com/watch?v=PNp7Vvu8jg>
4. In a 1.5 mL RNase-free microcentrifuge tube, add 40 µL of Reagent B4 (SVD-4-B4) stock solution. Add 4 µL of 1X Reagent A (SVD-3) stock solution and mix by pipetting up and down 5-10 times. Incubate in a 37 °C water bath for 1 hour. After incubation, keep the A-B solution at room temperature until use. Note: The A-B solution must be used within 3 hours.
5. Add the entire volume (200 µL) of LNP-mRNA mixture (step 3) to the A-B solution. Mix by pipetting up and down 5-10 times. Incubate the mixture on a shaker (300 rpm) at room temperature for 1 hour.
6. Add 156 µL of PBS and gently mix by pipetting 3-5 times, store at 4 °C until use. **Note: It should be used in 24 hours.**
7. Transfer 10 µL, 40 µL, 80 µL, 160 µL (~100, 400, 800, 1,600 ng) of the final LNP-mRNA stock solution into each well of 96-, 24-, 12-, 6-well plate containing 0.1 X10<sup>6</sup>, 0.5 X10<sup>6</sup>, 1.0 X10<sup>6</sup>, 2.5 X10<sup>6</sup> human PBMC cells.

## Notes

- Please maintain sterile and RNase free conditions at each step.
- The vortex speed should be around 2000 RPM.
- Please ensure that the lipid mixture (SVD-1) is not subjected to more than two freeze-thaw cycles to maintain its performance.

## Warnings

**This product is for research use only.** Reverse engineering, patent filing, resale, or any other form of commercialization is strictly prohibited. Any intended commercial use of this kit must be authorized in advance by SUNVAX MRNA Therapeutics.

Human CD4 T cell targeted SV104 Delivery™ Kit information and Supporting Data

Table 1 The Characterizations of human CD4 T cell targeted LNP-mRNA generated by SV104 Delivery™ Kit

|                 | EE (%)   | Size (nm) | PDI       |
|-----------------|----------|-----------|-----------|
| SV104 Delivery™ | 96.5±0.9 | 97.8±2.6  | 0.17±0.04 |

The kit exhibits consistently high encapsulation efficiency (>95%), generate nanoparticles with a uniform average size of approximately 98 nm, and maintain a low polydispersity index (PDI).

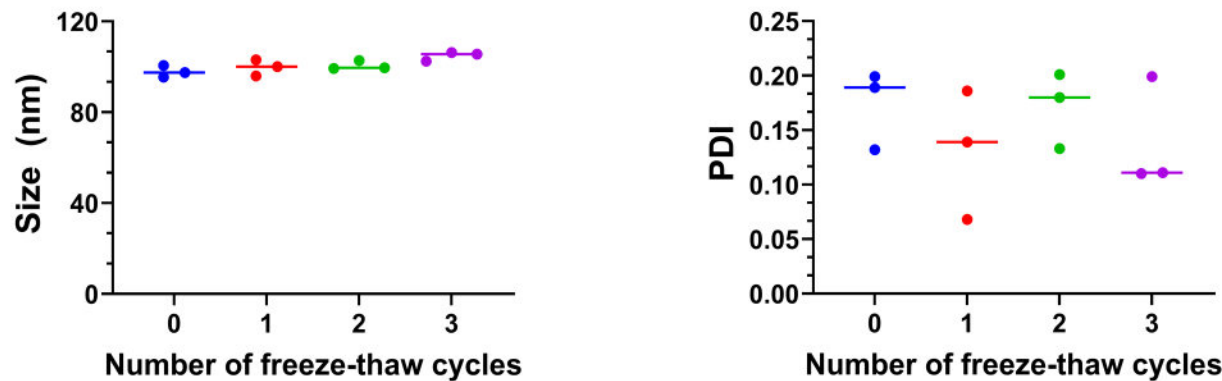


**Figure 1.** Stability of human CD4 T cell targeted LNP-mRNA generated by SV104 Delivery™ Kit. The human CD4 T cell targeted LNP-mRNA underwent three freeze–thaw cycles. Shown are nanoparticle size (left) and polydispersity index (PDI) (right).

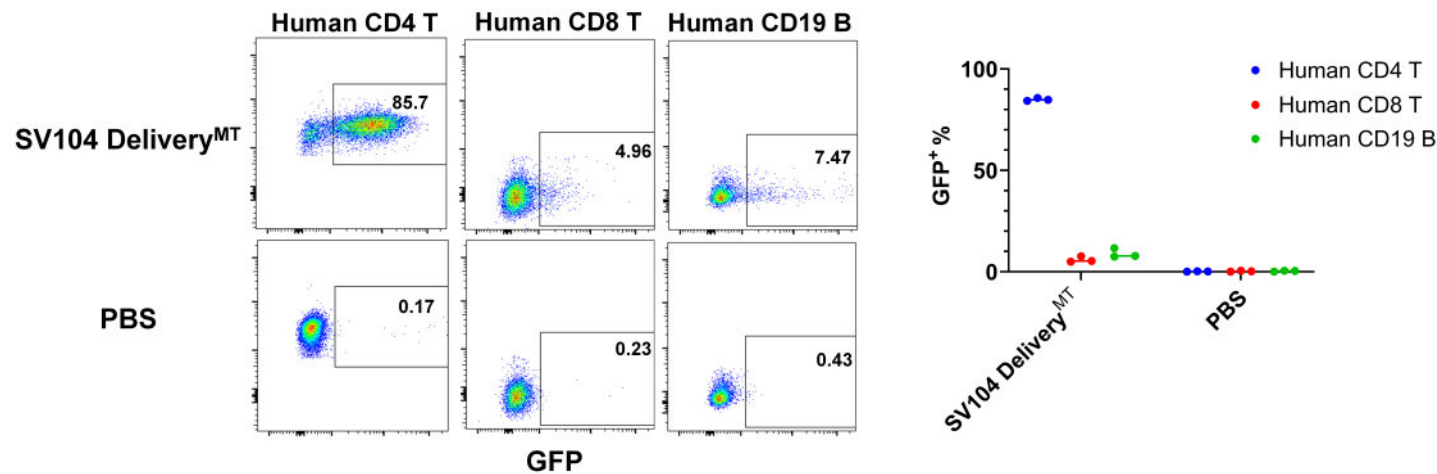


**Figure 2.** The SV104 Delivery™ kit demonstrated strong transfection efficacy and cell type specificity in human CD4 T cells. *In vitro* transfection efficacy: human CD4 T cells were transfected with mRNA encoding eGFP using SV104 Delivery™ kit as indicated. Shown are FACS plots (A) and GFP<sup>+</sup> cells percentage (B) one day post-transfection determined by flow cytometer.