Vivofectamine Delivery Solutions provide easy access to advanced LNP technologies for academic research

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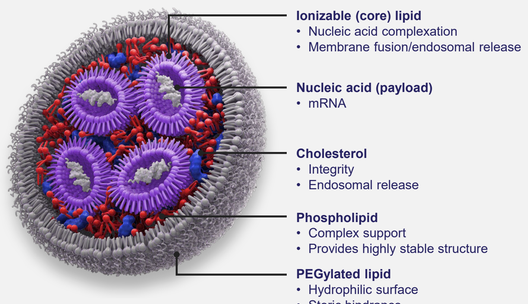
Vivofectamine chemically diverse LNP library

The rapidly expanding use of mRNA as a therapeutic modality has prompted the development of novel delivery methods, such as lipid nanoparticles (LNPs). Leveraging over 30 years of experience with lipid-based delivery technologies, we have curated LNP solutions that efficiently deliver mRNA *in vivo*. Here, we present two offerings tailored for academic researchers under Invitrogen™ Vivofectamine™ Delivery Solutions. The first formulation is Invitrogen™ Vivofectamine™ VF232 Liver LNP Composition in Ethanol, which is designed for intravenous delivery to the liver. The second is Invitrogen™ Vivofectamine™ VF233 IM LNP Composition in Ethanol. This reagent is intended for intramuscular (IM) delivery, particularly for prophylactic and cancer vaccine research. We have evaluated these off-the-shelf, easy-to-use LNP formulations in terms of particle characterization and compared their *in vivo* performance to relevant clinical benchmarks.



Development and selection of Vivofectamine lipids

6,000+ ionizable lipids synthesized
- Chemically diverse for various application needs
- Biodegradable lipid structure design

2,000+ lipids screened *in vitro*
- Formation of stable LNPs
- *In vitro* efficiency and toxicity were evaluated

500+ lipids screened *in vivo*; 15 in nonhuman primates (NHPs)
- *In vivo* efficacy, organ targeting, and tolerability were tested
- Comparison to clinical benchmarks

Top 10+ application-specific lipids selected
- Detailed biological and biophysical characterization
- Tested in large animals (rats and/or NHPs)

Application areas

Vivofectamine reagents were specifically tailored for key applications in vaccine development and liver-targeting delivery.

Goals	Basic research	
Applications	Vivofectamine VF232 Liver LNP Composition in Ethanol Liver: intravenous (IV)	Vivofectamine VF233 IM LNP Composition in Ethanol Vaccine: intramuscular (IM)
Format	**LNP composition in ethanol** The premixed ethanol solution contains an ionizable lipid and helper lipids at an optimized ratio specific to the application.	
Models	The reagents have been tested in mice.	

Characterization of Vivofectamine mRNA-LNPs

mRNA-LNPs were formulated using a microfluidic device and frozen in a cryobuffer. Particle characteristics were analyzed before freezing and after 1 week at –80°C.

Product	Condition	Size (nm)	PDI*	Encapsulation efficiency
Vivofectamine VF232 Liver reagent	Fresh	68.2	0.11	98%
	Frozen/thawed	74.0	0.15	94%
Vivofectamine VF233 IM reagent	Fresh	88.1	0.09	94%
	Frozen/thawed	85.2	0.21	93%

* PDI = polydispersity index

Vivofectamine VF232 Liver reagent allows efficient and specific delivery of mRNA, siRNA, and sgRNA/mRNA combinations *in vivo*.

Protein expression

Efficacy: *in vivo* bioluminescence analysis
4 hours after IV delivery of firefly luciferase (*fLuc*) mRNA

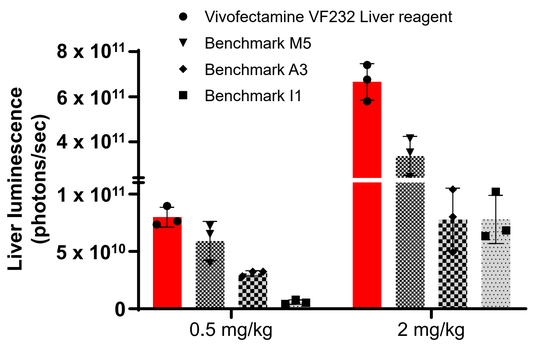


Figure 1. Bioluminescence analysis of *fLuc* mRNA-LNP in mice at 4 hr post-delivery indicates efficient protein expression relative to benchmarks. Vivofectamine VF232 Liver reagent was used to encapsulate an *fLuc* mRNA-LNP using a microfluidic instrument. The mRNA-LNP was dialyzed and injected intravenously into BALB/c mice at different doses. Three benchmark controls utilized ionizable lipids that are employed in clinical-stage mRNA liver therapies or FDA-approved siRNA liver therapy.

Protein knockdown

Efficacy: factor VII knockdown in serum
24 hours after IV delivery of FVII siRNA-LNP

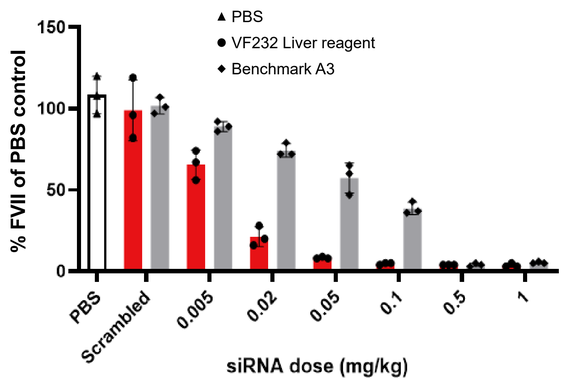


Figure 2. Factor VII colorimetric assay using siRNA-LNPs in mouse serum shows effective protein knockdown. siRNA-LNPs were formulated using Invitrogen™ Ambion™ *In Vivo* Factor VII (FVII) siRNA with Vivofectamine VF232 Liver reagent, followed by intravenous injection into BALB/c mice at different doses. Serum was collected 24 hr post-dosing, and FVII levels were analyzed using a colorimetric assay to determine knockdown efficiency. Benchmark A3 is an ionizable lipid in FDA-approved siRNA liver therapy.

Genome editing

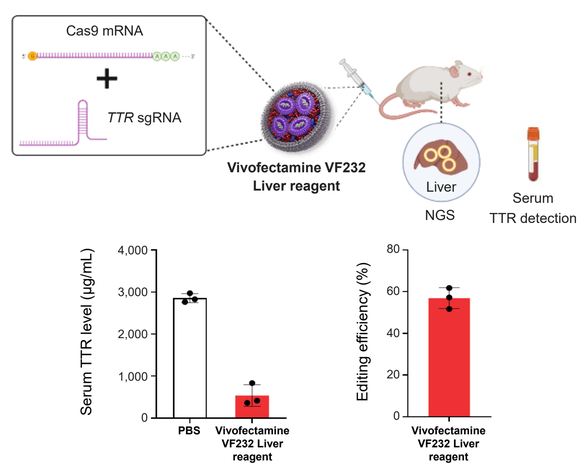


Figure 3. Delivery of Cas9-mRNA and sgRNA in LNPs shows efficient genome editing in mouse liver. Vivofectamine VF232 Liver reagent was used to co-encapsulate a Cas9 mRNA and sgRNA at a 1:1 weight ratio. A total dose of 3 mg/kg RNA was delivered intravenously to BALB/c mice. One week after injection, circulating TTR proteins were analyzed in serum by ELISA. The levels of genome editing in homogenized liver were tested using next-generation sequencing (NGS).

Vivofectamine VF232 Liver reagent shows good tolerability in mice.

Liver chemistry

Levels of liver enzymes in serum
24 and 48 hours after IV injection of 1 mg/kg mRNA-LNP

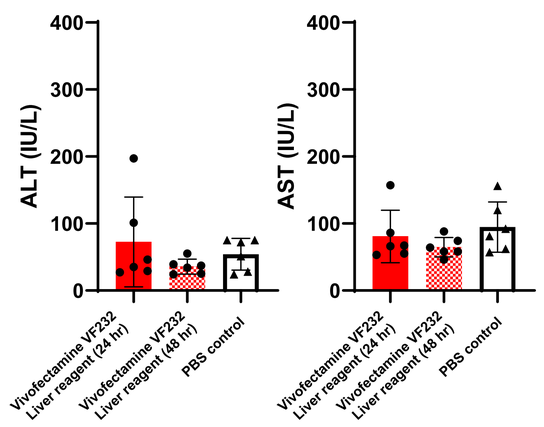


Figure 4. Liver enzymes at 24 and 48 hr post-IV injection of mRNA-LNPs show no elevation relative to PBS control in mice. Vivofectamine VF232 Liver reagent was used to encapsulate *fLuc* mRNA, and the mRNA-LNPs were injected intravenously into BALB/c mice at a dose of 1 mg/kg. For clinical chemistry analysis, serum was collected at 24 and 48 hr after delivery of the mRNA-LNPs. Levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were compared to a PBS-injected control.

Cytokine expression

Inflammatory cytokine response

36 cytokines | 2 time points

Unmodified mRNA was used as the positive control for induction of inflammation.

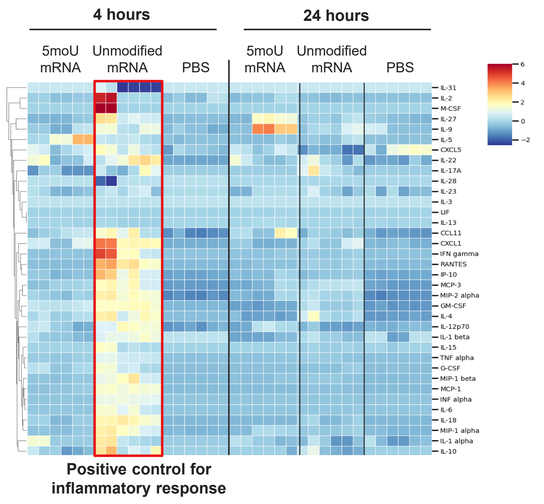


Figure 5. mRNA-LNPs injected into mice elicit low to no inflammatory response, as evidenced by a panel of 36 cytokines. Vivofectamine VF232 Liver reagent was used to encapsulate *fLuc* mRNA, and the mRNA-LNPs were injected into mice at a dose of 0.5 mg/kg. The serum was collected at 4 hr and 24 hr time points and analyzed using the Invitrogen™ ProcartaPlex™ Mouse Cytokine & Chemokine Panel.

Vivofectamine VF233 IM reagent shows effective immunization and desirable biodistribution in mice.

Immunization

Figure 7. Immunization of mice with *HA* mRNA-LNP shows similar levels of anti-HA IgG at 3 and 6 weeks relative to those elicited by benchmark lipids. H10N8 hemagglutinin (*HA*) mRNA encapsulated in Vivofectamine VF233 IM reagent was injected into BALB/c mice at a dose of 0.25 mg/kg. A booster injection was performed 3 weeks after the initial injection. Serum was collected 3 weeks after the initial injection and boost doses, and levels of anti-HA IgG were analyzed using ELISA. Benchmarks M102 and B315 are lipids used in commercial mRNA vaccines.

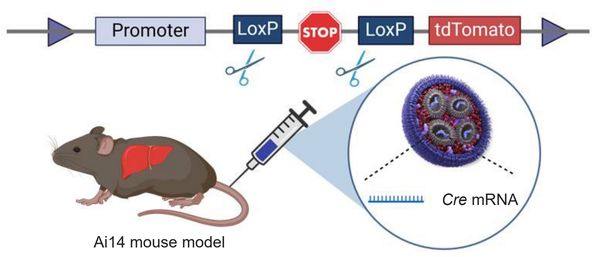
Biodistribution

Figure 8. Injection of *fLuc* mRNA-LNP into mice displays localized distribution of the luciferase protein in the muscle. *fLuc* mRNA was encapsulated in Vivofectamine VF233 IM reagent and injected into BALB/c mice at a dose of 0.25 mg/kg. Expression levels of the luciferase protein were analyzed *in vivo* **(top)** and *ex vivo* **(bottom)** by an IVIS Lumina III system 4 hr after injection. Benchmarks M102 and B315 are lipids used in commercial mRNA vaccines.

Visualization of delivery

LNP-targeted cells in the liver

tdTomato expression in Ai14 tdTomato reporter mice



Cre mRNA-LNP using Vivofectamine VF232 Liver reagent

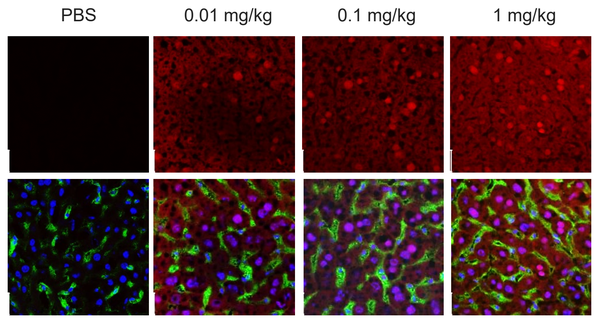


Figure 6. *Cre* mRNA-LNP injected intravenously into an Ai14 tdTomato reporter mouse model shows expression of tdTomato in the liver, even at a low dose. Livers were isolated, cryosectioned, immunostained, and visualized using confocal microscopy. The expression levels of tdTomato were compared to a PBS-injected control Ai14 group.

Summary

- ✓ Vivofectamine VF232 Liver reagent performs on par with benchmark ionizable lipids used in clinical-stage therapies.
- ✓ Vivofectamine VF232 Liver reagent provides efficient siRNA-mediated knockdown and CRISPR-Cas9–mediated knockout.
- ✓ Vivofectamine VF233 IM reagent induces an immune response at levels similar to clinical benchmark lipids.
- ✓ Vivofectamine basic research reagents demonstrate good tolerability in mice.

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