

Cytiva™ CD34+ HSC LNP kits

Discover and scale up your hematopoietic stem cell therapy using lipid nanoparticles

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Gene modified CD34+ hematopoietic stem cells (HSCs) have tremendous therapeutic potential in addressing various hematological disorders. HSCs are difficult to source and have a limited window of manipulation making this cell type challenging to work with and manufacture reliably. Current gene delivery methods such as electroporation can be harsh on cells, making it difficult to generate gene-modified cells with high viability and reach a yield sufficient for an effective therapeutic^{1,2}. Lipid nanoparticles (LNPs) enable gentle and highly efficient delivery of genetic material to HSCs and its scalability supports acceleration from discovery to preclinical and beyond.

The CD34+ HSC LNP kit is an off-the-shelf LNP reagent mix optimized for the delivery of messenger RNA (mRNA) or Cas9 mRNA/sgRNA into CD34+ HSCs. This kit is available in two sizes: 100 µL for the NanoAssemblr® Spark™ and 2 mL for the NanoAssemblr® Ignite™.

LNPs are prepared either using the Spark™ or Ignite™ instruments with respective NxGen™ Cartridges. This non-viral delivery method can be seamlessly integrated into any standard CD34+ HSC workflow using an established protocol with cells obtained from human cord blood or mobilized peripheral blood. With these kits researchers can establish a clinically-relevant and scalable method at the discovery and preclinical scale for *ex vivo* gene delivery and editing.

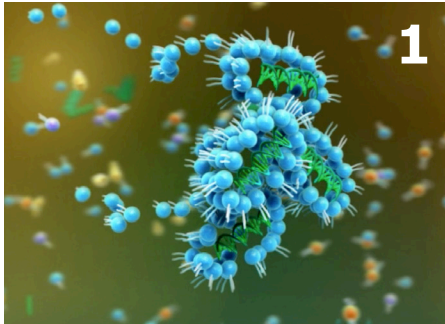
Why Use the CD34+ HSC LNP kit?

- Deliver RNA with high transfection efficiency using an optimized proprietary LNP formulation*
- Achieve dose-dependent high efficiency gene knockout
- Maintain high cell viability, yield and differentiation potential post-editing
- Scale up your *ex vivo* cell therapy research from discovery to preclinical on the NanoAssemblr platform
- Optimized and validated for downstream CD34+ HSC cell manufacturing workflow

*LNP compositions in the research-use-only kits include ionizable lipid excipients that can be licensed for future clinical evaluation.



RNA-LNPs Leverage Endogenous Cellular Uptake Mechanisms



Lipid nanoparticles contain ionizable lipids, which at low pH mediate efficient encapsulation of RNA at low pH



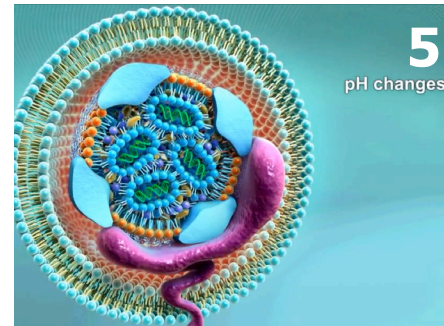
The RNA-lipid core is surrounded by helper lipids, cholesterol and stabilizers to form the RNA-LNP complex



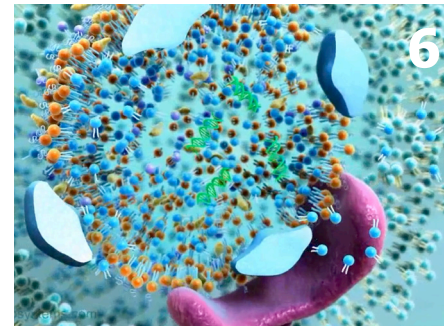
Once formed, RNA-LNPs are neutral at physiological pH, eliminating a source of toxicity present in other RNA delivery methods



RNA-LNP mimic low density lipoproteins (LDL) and are then taken up through receptor mediated endocytosis



Once in the endosome, ionizable lipids in RNA-LNP respond to low pH and become cationic



The cationic lipids in the RNA-LNP interact with anionic lipids in the endosome and release the RNA into the cytoplasm

CD34+ HSC LNP kits

Consistent LNP Size and RNA Encapsulation Efficiency

Lipid nanoparticles formulated on the NanoAssemblr Spark (Discovery) and Ignite (Preclinical) instruments yield consistent size, polydispersity index (PDI), and encapsulation efficiency.

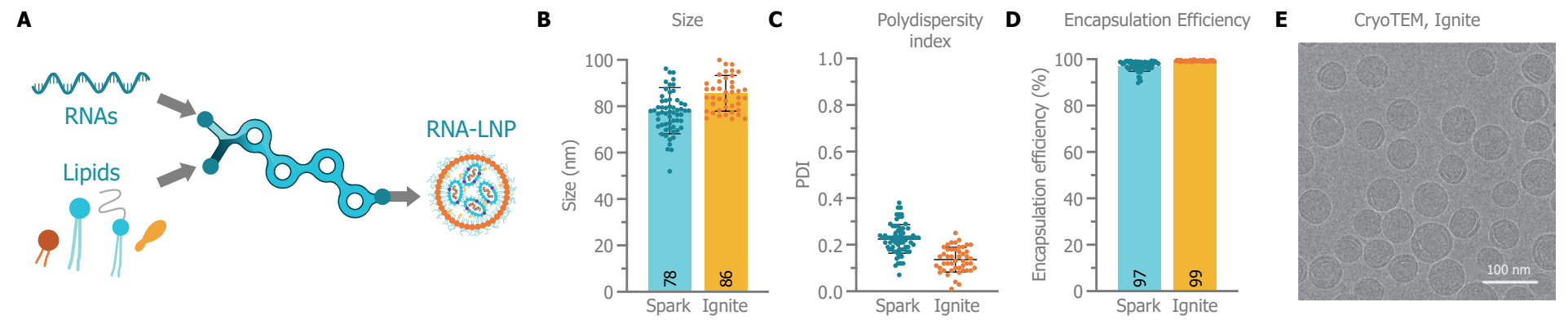


Figure 1. A) Schematic diagram of the LNP formulation process using the NanoAssemblr technology. Physicochemical data **B, C)** Size and PDI, **D)** Encapsulation efficiency, and **E)** Cryo-TEM images of CRISPR Cas9 mRNA and sgRNA-loaded LNPs produced on the Ignite (preclinical scale) instruments.

Optimized LNP Formulation for CD34+ HSCs

Accelerate your research and development with an optimal LNP composition designed using our [proprietary lipid library](#). LNP composition significantly affects CD34+ HSC genome editing efficiency.

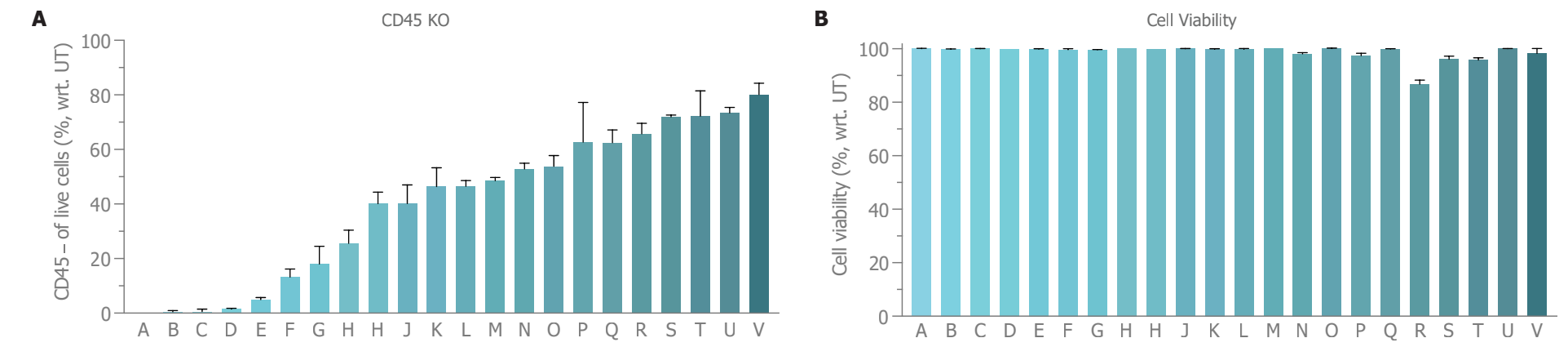


Figure 2. A) CD45 knockout efficiency using various LNP compositions. **B)** Cell viability as analyzed by flow cytometry.

Dose Dependent Gene Knockout of CD45

Achieve dose-dependent, highly efficient genome editing of CD34+ HSCs sourced from human cord blood and human mobilized peripheral blood using the CD34+ HSC LNP kit, 100 µL on the Spark and CD34+ HSC LNP kit, 2 mL on the Ignite instrument.

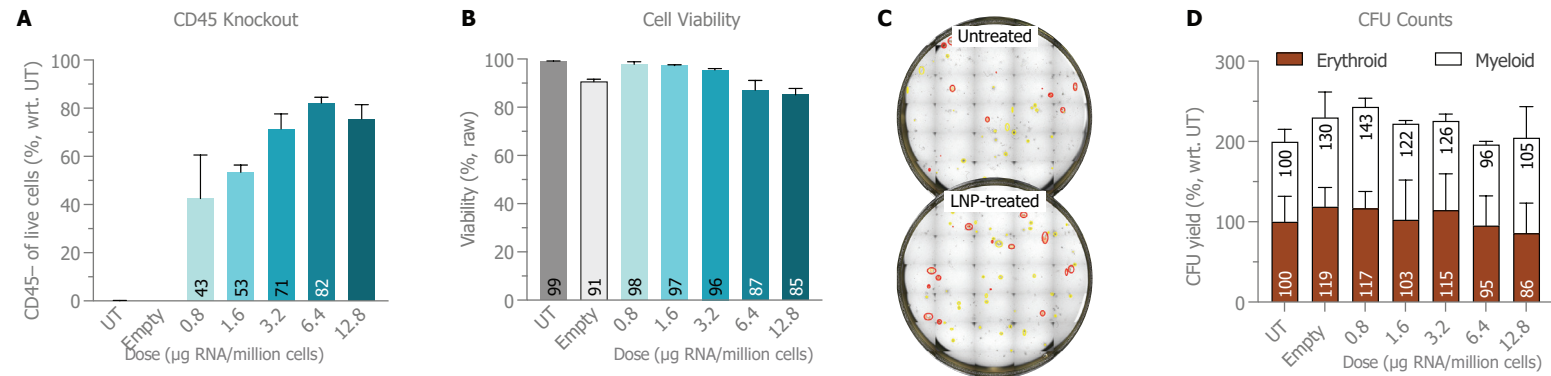


Figure 3. A) CD45 dose response of CRISPR-Cas9 mRNA and sgRNA LNPs produced on the NanoAssmblr Spark instrument. **B)** Corresponding cell viability. **C)** Representative images of untreated and CRISPR RNA-LNP treated CFU plates. **D)** Normalized erythroid and myeloid colony yields of untreated (UT), empty LNP treated, and CRISPR RNA-LNP treated samples.

Cell Proliferation and Viability Following LNP Treatment

LNP-treated HSCs show high cell proliferation and similar viability compared to untreated cells.

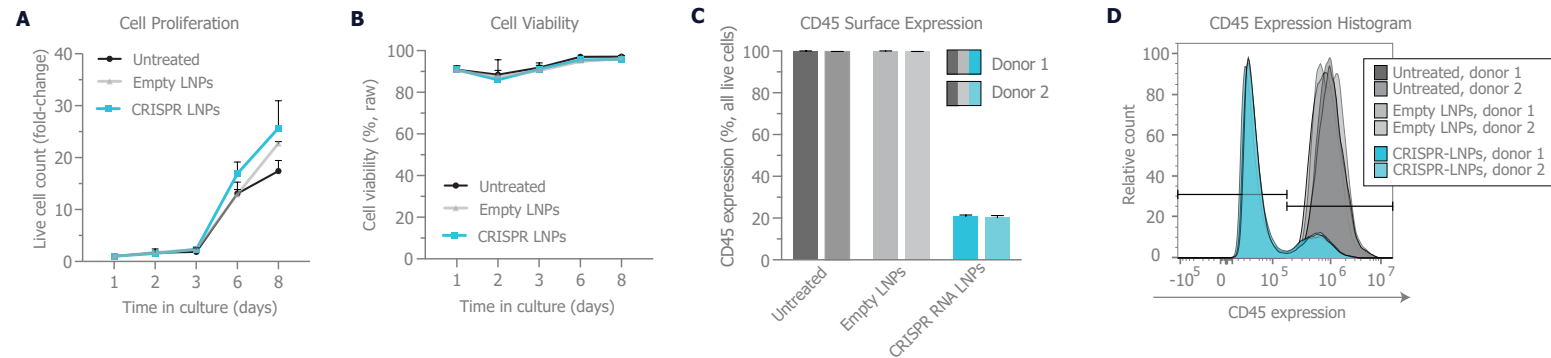


Figure 4. A) Cell proliferation and **B)** viability monitored for over 1 week after LNP-mediated CD45 targeted CRISPR-Cas9 gene editing of HSCs. **C)** LNP-mediated gene editing compared across n = 2 donors using flow cytometry via CD45 surface expression analysis and **D)** corresponding histogram.

HSC Cryopreservation and Freeze-Thawing Post LNP Treatment

Cryopreservation of LNP-treated HSCs showed high cell viability and yield. Furthermore, cells retained their desirable phenotype distribution while maintaining CD45 knockout efficiency.

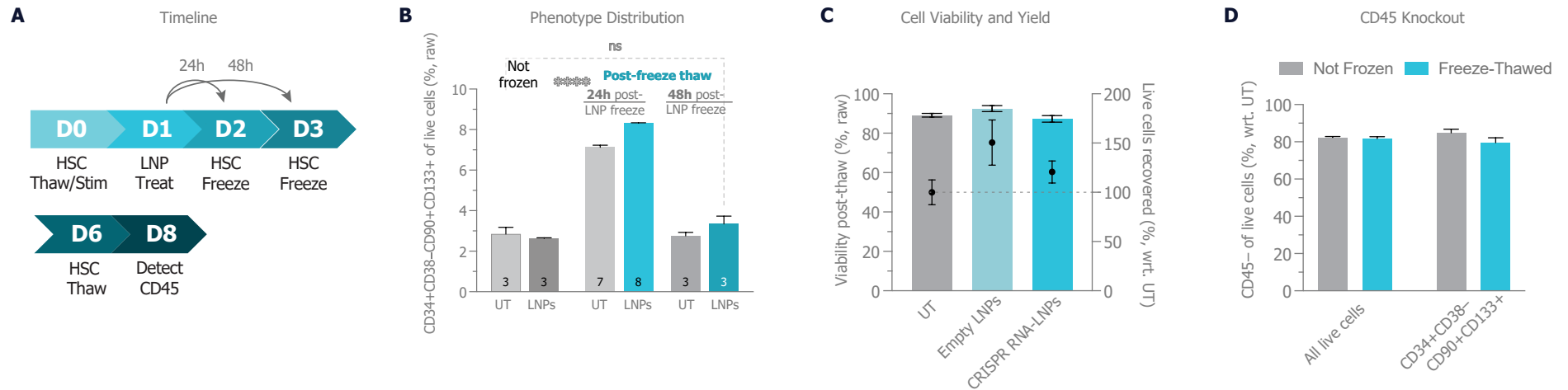
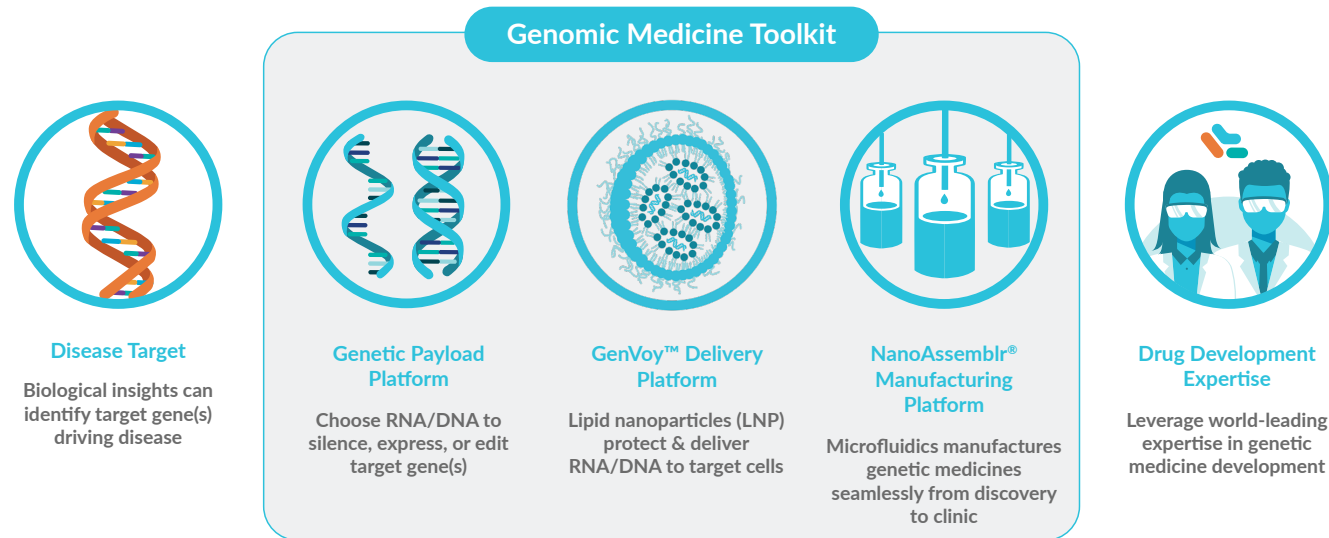


Figure 5. A) Schematic diagram of the freeze-thaw (F/T) cycles of HSCs. **B)** Post-thaw, or non-frozen long-term repopulating HSC phenotype levels, at either 24h or 48h post-LNP treatment freeze time points. **C)** Cell viability and live cell recovery post 24h-LNP treatment F/T cycle. **D)** CD45 knockout efficiency observed in freeze-thawed and fresh LNP-treated HSCs in total live cells and engraftable phenotype (CD34+ CD38- CD90+ CD133+).

Lipid Nanoparticles Empower Genomic Medicine Development

The genomic medicine toolkit comprises of GenVoy™ LNP delivery reagents, NanoAssemblr manufacturing platform and technical expertise enabling researchers to develop genetic vaccines, gene therapies and cell therapies.



Easily Integrated into Standard CD34+ HSC Cell Culture Protocols

The CD34+ HSC LNP kits can be easily integrated into a CD34+ HSC workflow for both mRNA-based gene expression and CRISPR/Cas gene editing applications. A general workflow is outlined below.

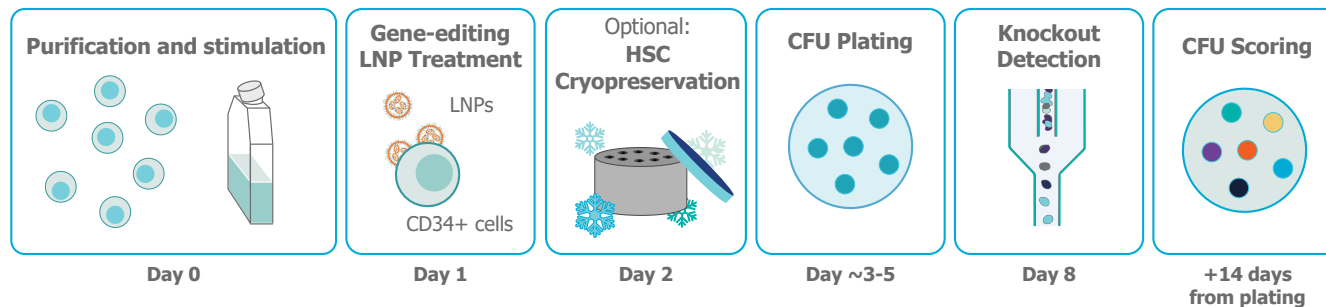


Figure 6. Schematic diagram of the LNP treatment and HSC cell culture workflow. LNP production may be decoupled from the cell culture workflow, allowing greater flexibility and simplicity of workflow.

Scale Up of Cell Culture and RNA-LNP Production

The CD34+ HSC LNP kits are available on the NanoAssemblr Spark and Ignite instruments. Reagents are also available for scale up, process development and GMP manufacturing through [BioPharma Services](#).



*Treatment does likely to fall between 2–6 µg RNA/million cells

Scale up from discovery to preclinical with a peace of mind. The NxGen NanoAssemblr platform enables robust and equivalent performance of the CD34+ HSC LNP kit, 100 µL on the Spark instrument to the CD34+ HSC LNP kit, 2 mL on the Ignite instrument. These data demonstrate the seamless scalability from discovery to preclinical studies.

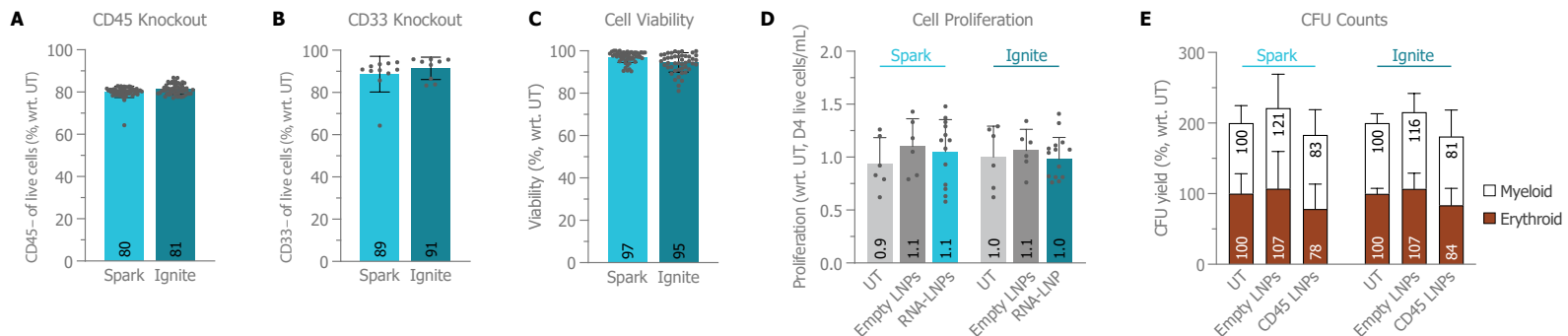
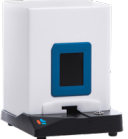
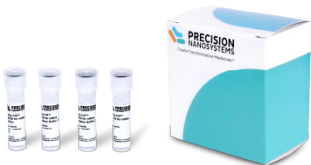


Figure 6. **A)** CD45 knockout and **B)** CD33 knockout of the total live cell population, as well as **C)** corresponding cell viability. A–C includes >20 unique experiments with >4 unique donors. **D)** Untreated, empty-LNP and CRISPR-Cas9 RNA loaded LNP treated HSCs were assessed for proliferation using an automated cell counter. **E)** Untreated, empty-LNP and CRISPR-Cas9 RNA loaded LNP treated HSCs were assessed for clonogenicity using an automated CFU counter, 5 independent experiments with Spark and 2 independent experiments with Ignite.

Ordering Information

CYTIVA CD34+ HSC LNP KITS		PRODUCT CODE	INCLUDES
	Cytiva™ CD34+ HSC LNP kit, 100 µL	1003000	Lipid mix, 100 µL Formulation buffer type 1, 400 µL Dilution buffer type 1, 1.5 mL Apolipoprotein-E3 (ApoE), 100 µg
	Cytiva™ CD34+ HSC LNP kit, 100 µL with cartridges	1004000	Lipid mix, 100 µL Formulation buffer type 1, 400 µL Dilution buffer type 1, 1.5 mL Apolipoprotein-E3 (ApoE), 100 µg 5 NanoAssemblr Spark Cartridges
	Cytiva™ CD34+ HSC LNP kit, 2 mL	1005000	Lipid mix, 2 mL Formulation buffer type 1, 6 mL Dilution buffer type 1, 100 mL (10X) Cryopreservation buffer type 2, 6mL Apolipoprotein-E3 (ApoE), 500 µg
INSTRUMENTS, CARTRIDGES AND ACCESSORIES		PRODUCT CODE	INCLUDES
	NanoAssemblr® Ignite™ and Ignite+™	NIN0001 1001413	1 Instrument 2 Sample Switch Arms 2 Cartridge Adapters
	Ignite™ NxGen™ Cartridges	NIN0061 NIN0062	100 pack 200 pack
	Ignite™ and Ignite+™ NxGen™ 500 Cartridges	1001397 1001398	50 pack 100 pack
	NanoAssemblr® Spark™	NIS0001	1 Instrument 1 Power Supply (Worldwide) 1 One-year Warrant
	Spark™ Cartridges	NIS0009 NIS0013	20 Pack 80 Pack

CYTIVA RNA DELIVERY LNP KIT		PRODUCT CODE	INCLUDES
	GenVoy-ILM™ T Cell Kit for mRNA, Spark	1000701	1 Kit
	GenVoy-ILM™ T Cell Kit for mRNA, Ignite, 3 mL and 6 mL	1001144 1001161	1 Kit

References

1. Vavassori V, Ferrari S, Beretta S, et al. Lipid nanoparticles allow efficient and harmless *ex vivo* gene editing of human hematopoietic cells. *Blood*. 2023;142(9):812-826. doi:10.1182/blood.2022019333
2. Kitte R, Rabel M, Geczy R, et al. Lipid Nanoparticles (LNPs) outperform Electroporation in mRNA-based CAR T cell Engineering. *Molecular Therapy: Methods & Clinical Development*. 2023. doi: <https://doi.org/10.1016/j.omtm.2023.101139>

About Precision NanoSystems (Now Part of Cytiva)

Precision NanoSystems is a global leader of innovative solutions for the discovery, development, and manufacture of genomic medicine-based gene and cell therapies, small molecules and protein-based drugs, rapidly taking ideas to patients. Precision NanoSystems ULC is now part of Cytiva.

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