PreciGenome

Product Catalog

NanoGenerator®
Nanoparticle Synthesis for LNP & PLGA

Aqueous Phase

Microfluidic Mixing

Nanoparticles

Solvent Phase
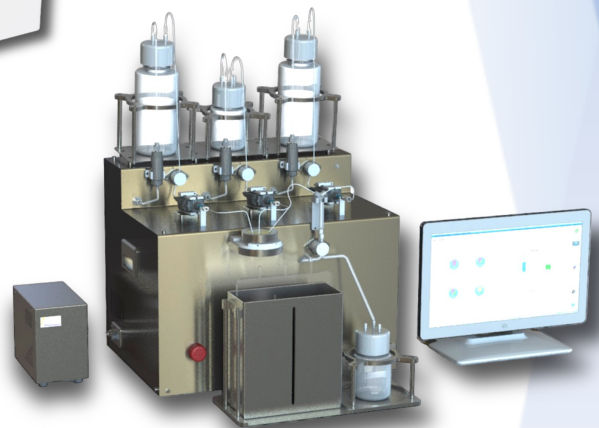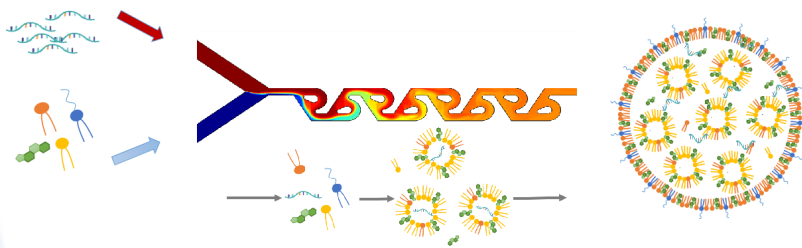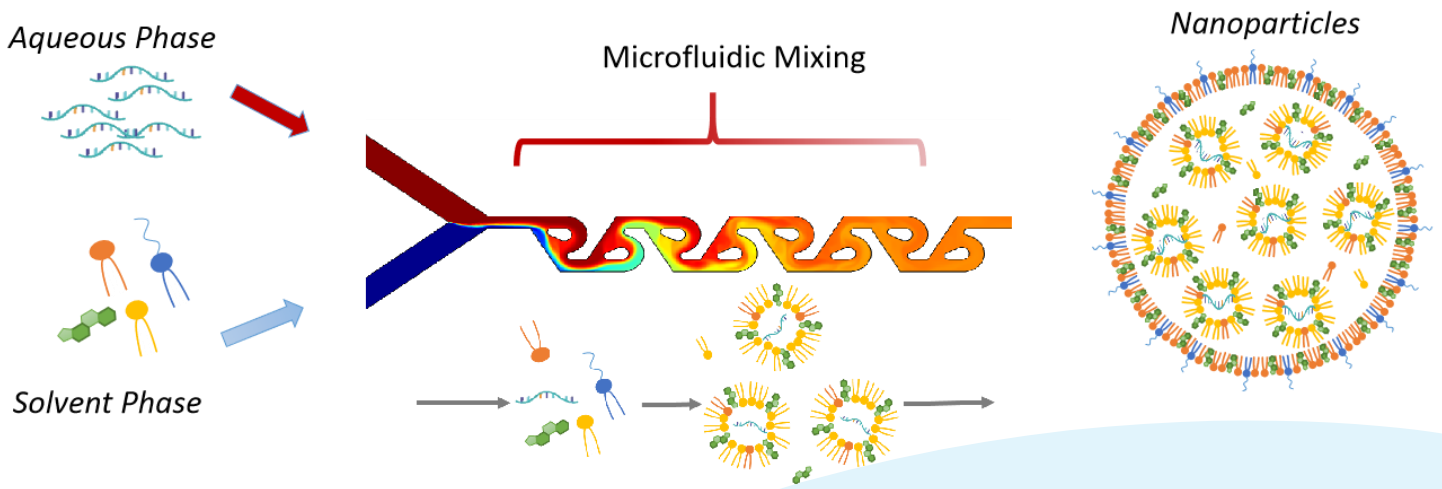
Nanoparticle synthesis via microfluidic mixing has superior control of size, homogeneity, and repeatability to conventional batch synthesis methods. Two streams, aqueous and solvent, meet in a narrow mixing channel, forming nanoparticles with payload encapsulated.

PreciGenome's NanoGenerator® platform uses pressure-based micro-fluidics for reliable nanoparticle synthesis at several production scales. It has been widely used to produce various types of nanoparticles, such as lipid nanoparticles (LNPs), liposomes, PLGA nanoparticles, etc.

Microfluidic Mixing System

- Controllable particle size
- Low PDI
- High encapsulation efficiency
- High reproducibility

System Benefits

High Performance & Efficiency

- Tunable size (40-200 nm)
- Low PDI
- High encapsulation efficiency

Open Platform

- Reagents
- Microfluidic chips

Scalable Throughput

- Scalable output from 100uL to >20L

Payloads

- mRNA/siRNA/other RNA
- DNA
- Proteins and peptides
- Small molecule drugs
- Other payloads

Simple Operation

- Easy setup
- Compact size
- Intuitive UI w/ touchscreen

Cost Effective

- Affordable configuration
- Low cost consumables

Custom design &OEM

- Research collaboration
- Custom design
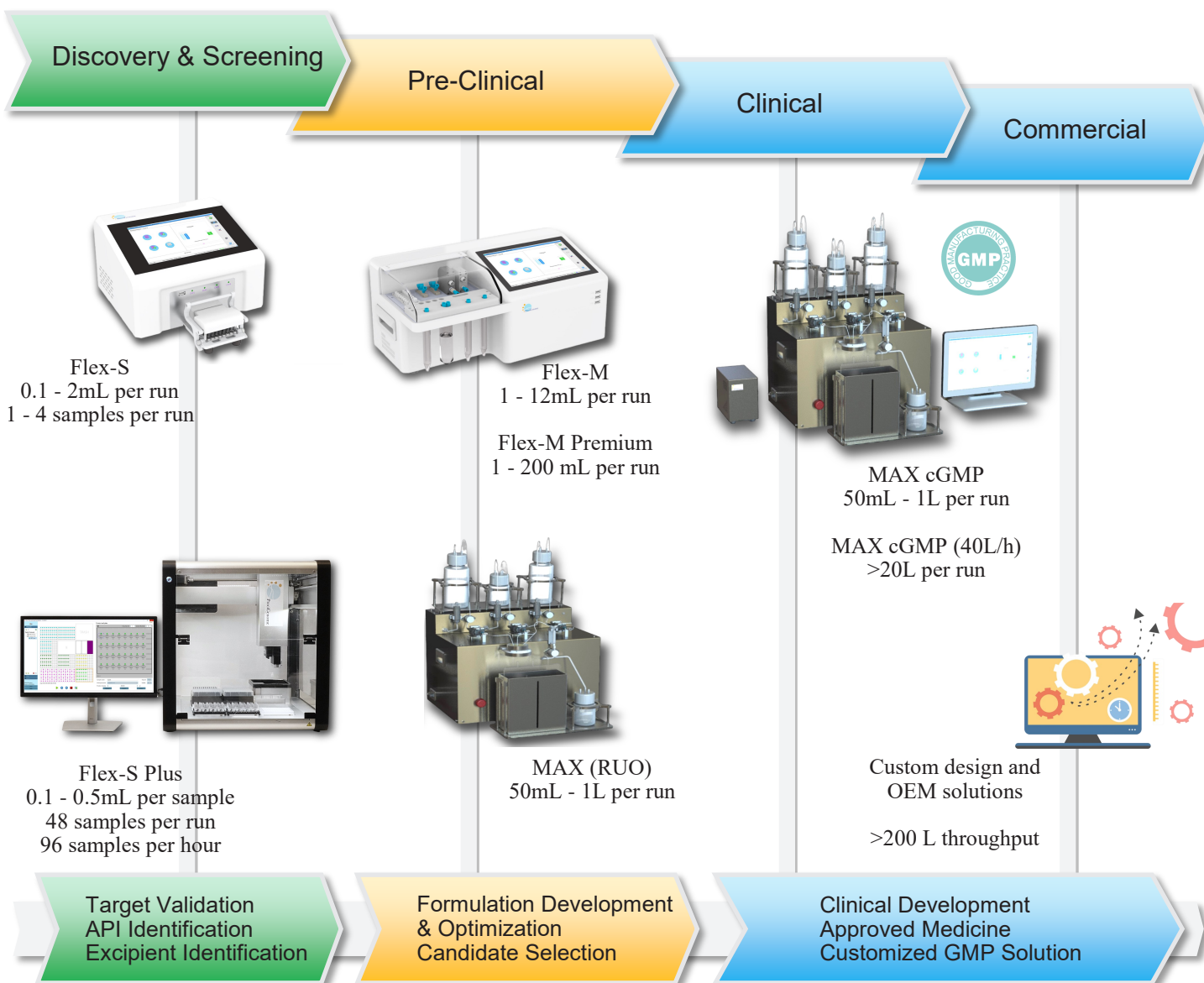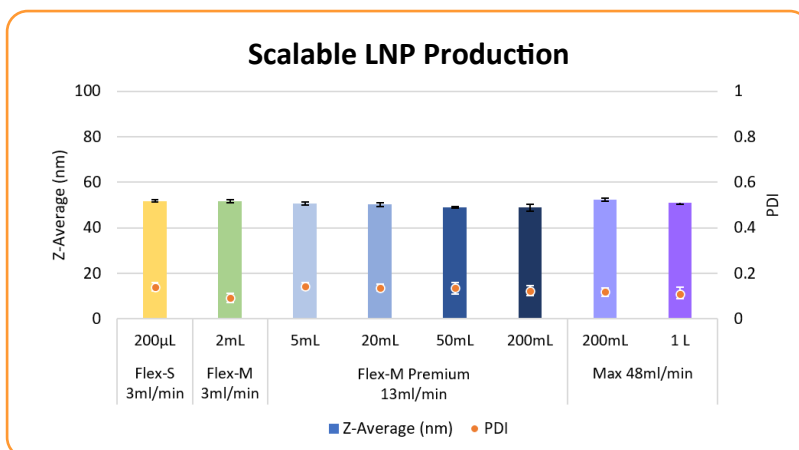- OEM & Contract manufac-turing

Path from Discovery to Commercialization

PreciGenome

NanoGenerator® offers controllable and repro-ducible mixing conditions, ensuring the accurate synthesis of LNPs through its scalable architec-ture found in the entire NanoGenerator® prod-uct line. Options are available for all production stages, allowing seamless transfer of crucial pro-cess parameters and guaranteeing consistent critical quality attributes (CQAs). LNPs produced from NanoGenerator® may be used for a wide range of applications, such as vaccine develop-ment, gene therapy, cell therapy, etc.

Scalable LNP Production

Z-Average (nm) / PDI

| 200μL | 2mL | 5mL | 20mL | 50mL | 200mL | 200mL | 1 L |
| Flex-S 3ml/min | Flex-M 3ml/min | | Flex-M Premium 13ml/min | | | Max 48ml/min | |

■ Z-Average (nm) ● PDI

Discovery & Screening

Pre-Clinical

Clinical

Commercial

GMP

Flex-S
0.1 - 2mL per run
1 - 4 samples per run

Flex-M
1 - 12mL per run

Flex-M Premium
1 - 200 mL per run

MAX cGMP
50mL - 1L per run

MAX cGMP (40L/h)
>20L per run

Flex-S Plus
0.1 - 0.5mL per sample
48 samples per run
96 samples per hour

MAX (RUO)
50mL - 1L per run

Custom design and
OEM solutions

>200 L throughput

| Target Validation
API Identification
Excipient Identification | Formulation Development
& Optimization
Candidate Selection | Clinical Development
Approved Medicine
Customized GMP Solution |

NanoGenerator® Flex-S
Nanoparticle Synthesis System

NanoGenerator® Flex-S

The NanoGenerator® Flex-S is designed for small scale production. It may run 1 to 4 samples at once, each sample from 0.1 to 0.5 ml. The throughput volume is therefore 0.1 to 2 ml per run, saving over 80% in reagent cost versus larger volumes and perfect for formulation screening and early discovery.

Even smaller output volumes (<0.1 ml per run), custom total flow rate, and custom flow rate ratio are attainable at special request by recipe optimization.

Model	NanoGenerator® Flex-S
Mixing Cartridge	CHP-MIX-4
Throughput	0.1 to 2 ml per run. 1 to 4 samples per run.
Total Flow Rate	3 ml/min, 4 ml/min
Flow Rate Ratio (W:O)	3:1
Size Range	40 to 200 nm
PDI	0.05 to 0.2
Encapsulation Efficiency	85-95%
Payloads	DNA, mRNA, siRNA, protein, small molecules

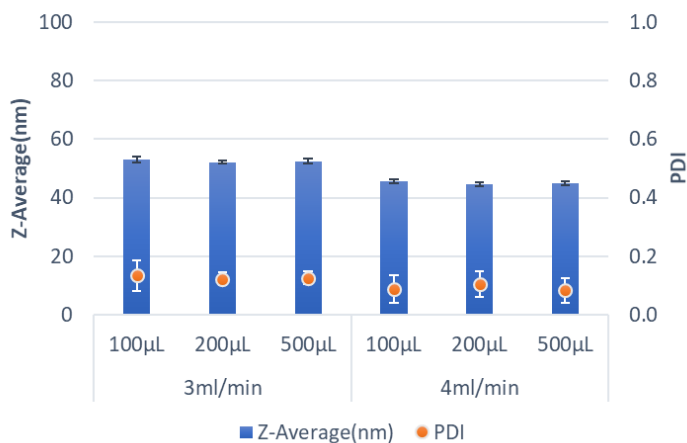## eGFP mRNA LNP Delivery to Jurkat Cells
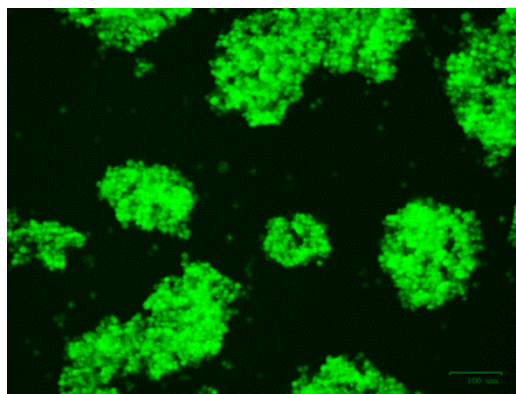
Jurkat Cells transfected with Formulation #9. Green fluorescence image at 48 hours post transfection.

Example of Formulation Screening by Flex-S

Screening Panel			LNP Characterization			Cell Study
Formulation	Ionizable Lipid	N/P Ratio	Size (nm)	PDI	EE%	GFP expression
#1	40%	3.57	56.6	0.19	86%	+
#2	40%	5.35	79.9	0.246	84%	+
#3	40%	8	75.2	0.214	85%	++
#4	60%	5.35	128.5	0.13	81%	NA
#5	40%	5.35	62.8	0.186	90%	++
#6	40%	8	54.3	0.184	93%	++
#7	50%	8	79	0.155	88%	+
#8	50%	11	82.2	0.126	90%	NA
#9	50%	8	87.5	0.12	91%	+++

Flexible Parameters of Flex-S

Z-Average(nm) / PDI chart with x-axis: 100µL, 200µL, 500µL (3ml/min), 100µL, 200µL, 500µL (4ml/min)

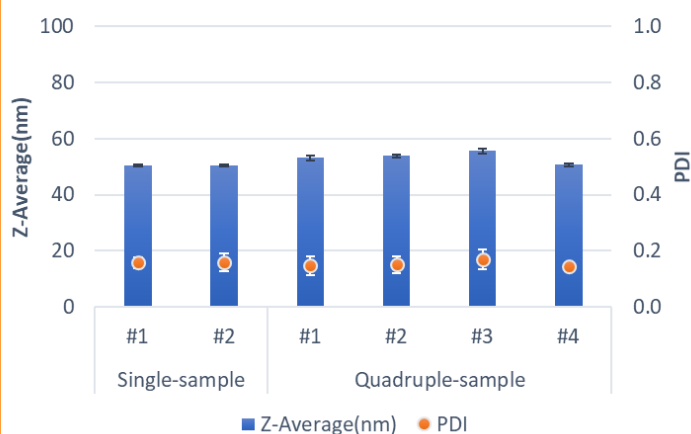Legend: ■ Z-Average(nm) ● PDI

New features of NanoGenerator® Flex-S:

- **More total flow rate settings.** Users can choose 3 or 4 ml/min to conduct LNP synthesis. Higher total flow rate generates smaller LNPs. Other total flow rate and flow rate ratio are attainable at special request. *LNP size and PDI also depend on other factors such as the payload and formulation choice.*

- **Output volume as low as 100 µl** is attainable by loading 75 µl aqueous samples (e.g. mRNA samples) and 25 µl lipid formulation.
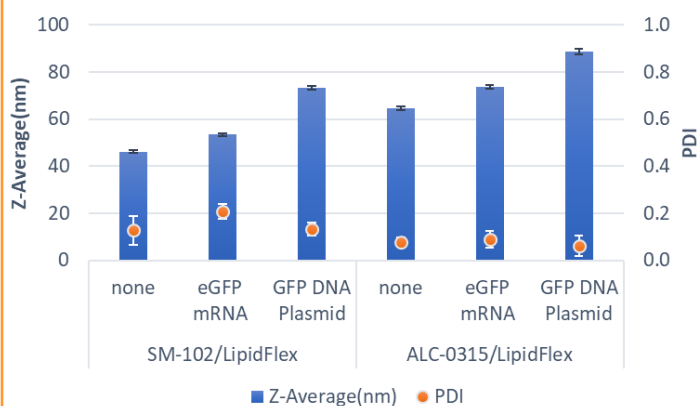
Multi-sample Synthesis by NanoGenerator® Flex-S:

- **10 seconds, 4 samples!** Users can enable multi-sample synthesis mode to conduct formulation screening. The screening time is as low as 10 seconds

- **Reliable screening results.** Using PreciGenome's advanced air-flow control technology, users can obtain reliable LNP results on both single- and multi-sample synthesis modes.

Multi-sample Synthesis Mode of Flex-S

Z-Average(nm) / PDI chart with x-axis: #1, #2 (Single-sample), #1, #2, #3, #4 (Quadruple-sample)

Legend: ■ Z-Average(nm) ● PDI

Nucleic Acid LNP Screening by Flex-S

Z-Average(nm) / PDI chart with x-axis: none, eGFP mRNA, GFP DNA Plasmid (SM-102/LipidFlex), none, eGFP mRNA, GFP DNA Plasmid (ALC-0315/LipidFlex)

Legend: ■ Z-Average(nm) ● PDI

- **Affordable formulation screening.** With the Nano-Generator® Flex-S, users can conduct formulation screening with minimal reagent consumption and reduced reagent cost.

- **Excellent batch-to-batch consistency.** Ease of operation and reliable components ensure consistent performance.

NanoGenerator® Flex-S Plus Nanoparticle Synthesis System

The Flex-S Plus System facilitates the rapid screening of nanoparticle formulations and early-stage mRNA candidates, offering a substantial increase in project efficiency.
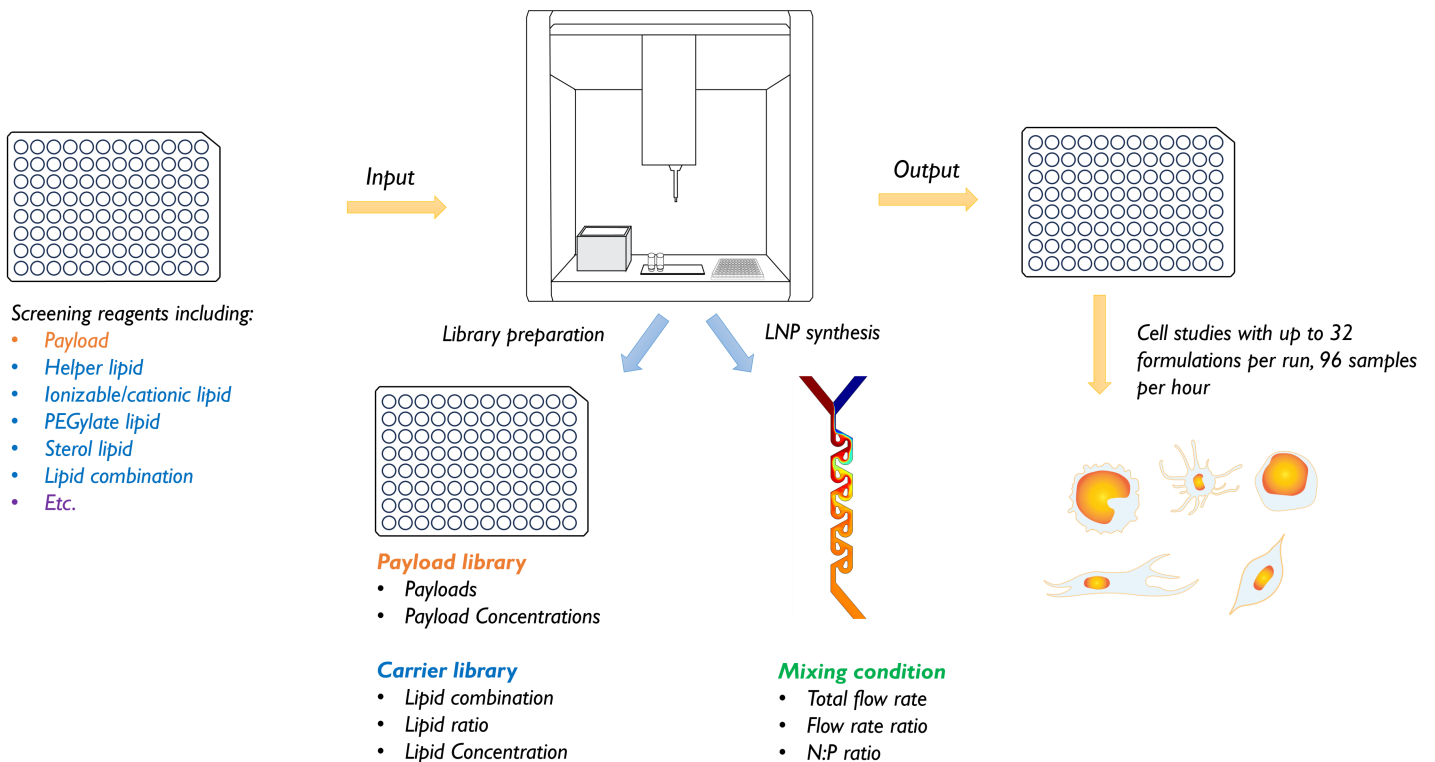
With a max throughput of 48 samples per run, 96 samples per hour, the Flex-S Plus greatly streamlines screening processes. It offers comprehensive automation of complex protocols, enabling users to concentrate on other laboratory duties.

The system also permits experimentation with as little as 25 µl of payload reagent (e.g. mRNA) while providing control over collection volumes. This allows users to optimize the use of valuable materials.

NanoGenerator® Flex-S Plus

Automated Screening Workflow

Screening reagents including:
- Payload
- Helper lipid
- Ionizable/cationic lipid
- PEGylate lipid
- Sterol lipid
- Lipid combination
- Etc.

Input

Library preparation

LNP synthesis

Output

Cell studies with up to 32 formulations per run, 96 samples per hour

Payload library
- Payloads
- Payload Concentrations

Carrier library
- Lipid combination
- Lipid ratio
- Lipid Concentration

Mixing condition
- Total flow rate
- Flow rate ratio
- N:P ratio

Formulation Screening & Discovery with High-throughput

Aqueous Phase

Microfluidic Mixing

Nanoparticles

Solvent Phase
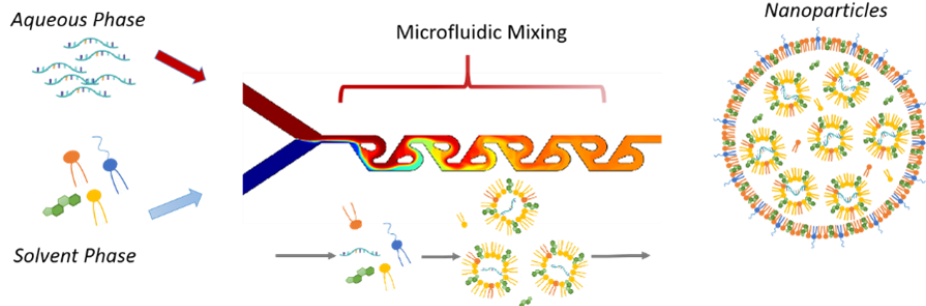
Cationic/ionizable lipid	
Helper lipid	
Cholesterol	
PEGylated lipid	

mRNA	DNA Plasmid
SiRNA	Cas9mRNA + sgRNA

Model	NanoGenerator® Flex-S Plus
Mixing Cartridge	CHP-MIX-4
Throughput	0.1 to 0.5 ml per sample.
Sample per run	(1 - 12) × 4 per run Up to 96 samples per hour
Library Preparation	Optional
Total Flow Rate	3 ml/min, 4 ml/min
Flow Rate Ratio (W:O)	3:1
Size Range	40 to 200 nm
PDI	0.05 to 0.2
Encapsulation Efficiency	85-95%
Screening factors	Lipids, Payloads, N:P ratio, etc.

Multi-sample Screening by NanoGenerator® Flex-S Plus:

Example screening factors with the Flex-S Plus include payloads, carrier formulation, total flow rate, flow rate ratio, N:P ratio, Lipid concentration, and payload concentration. Precise control of parameters ensures consistent CQAs, guaranteeing speed, cost-effectiveness, and reliability at every stage of the experiment.

Flex-S Plus consistency

Lipid formulation screening

Application — 32 sample Screening by NanoGenerator® Flex-S Plus:

The following shows an application study of 32 sample screening by NanoGenerator® Flex-S Plus with total flow rate of 3ml/min and flow rate ratio of 3:1. Each cell in the heatmap indicates a unique LNP sample with a specific lipid formulation or a specific N:P ratio. The following heatmap offers an insight of the physical properties (size & PDI) of 32 kinds of different LNPs

32 sample screening — Size (nm)

54.9	45.4	45.5	55.5
54.4	47.6	45	46.2
54.7	46.5	47.4	62.3
54.4	48.2	51.9	57.3
42.6	47.8	44.8	51.2
41.1	50	46.9	48.2
62.8	48.5	46.7	74
56.6	50.7	52.1	59.2

40   80

32 sample screening — PDI

0.216	0.13	0.126	0.473
0.175	0.107	0.092	0.08
0.185	0.09	0.104	0.113
0.16	0.1	0.137	0.107
0.306	0.121	0.101	0.403
0.063	0.129	0.128	0.124
0.118	0.066	0.148	0.109
0.041	0.099	0.163	0.113

0.02   0.50

System Benefits

High Throughput & Efficiency

- Multiple sample (1/4/32) per run.
- Runtime <5 min for 4 samples, 48/96 samples per hour.

Automation

- Automated workflow
- Real-time data monitoring & recording
- Electronic batch records

High Yield

- Small reagent volume (minimum 100 µl) for each sample.
- Save up to 80% of RNA/lipid cost

Email: USSales@precigenome.com
Tel: +1-408-708-4602

Address: 2176 Ringwood Ave., San Jose, CA, USA
Visit us at www.precigenome.com/nanoparticle-synthesis

NanoGenerator$^{®}$ Flex-M
Nanoparticle Synthesis System
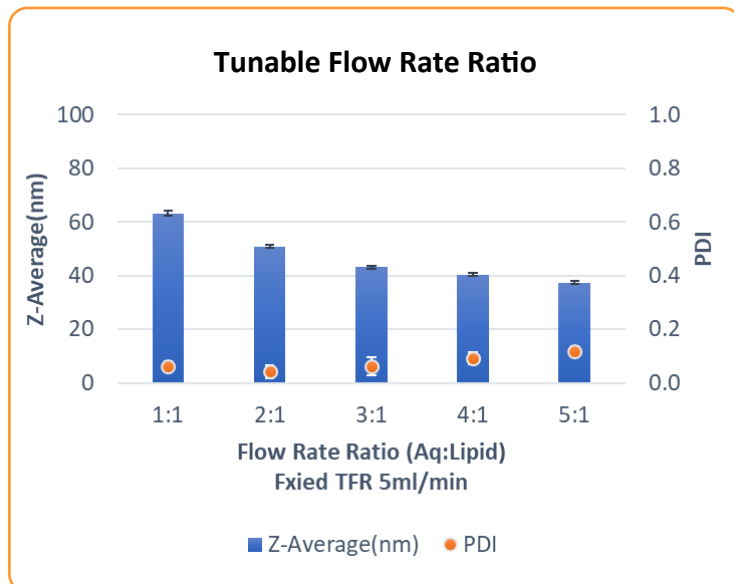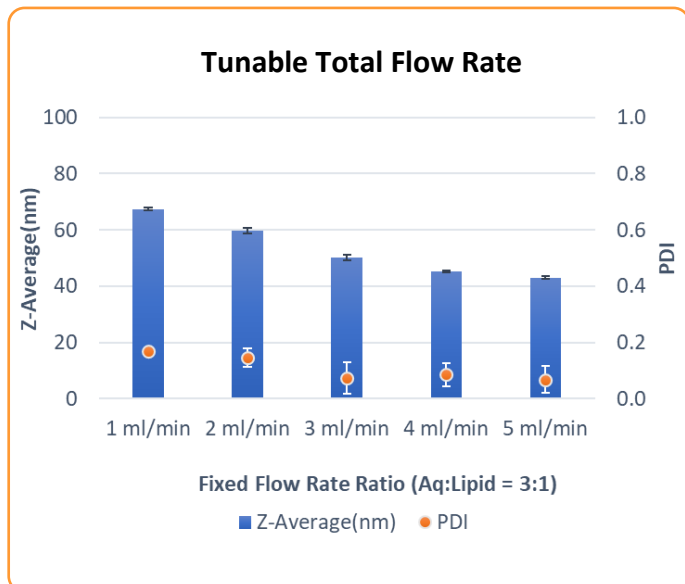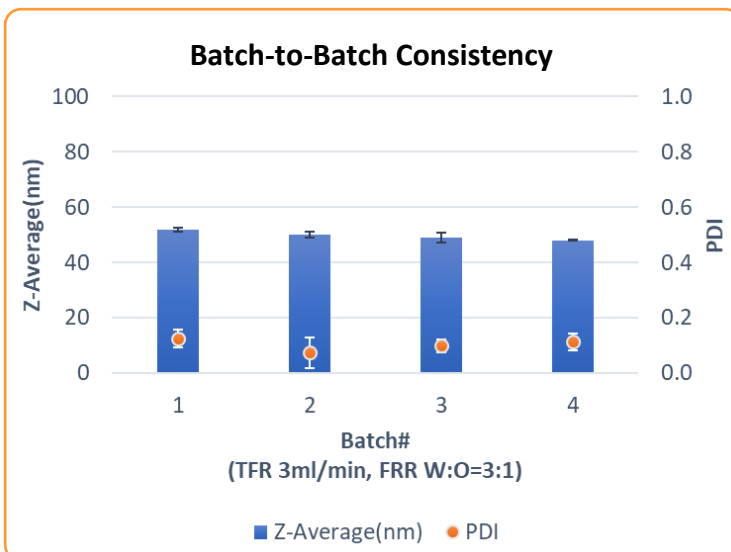
The NanoGenerator® Flex-M is designed for small to medium scale production. It has a throughput range from 1 to 12 ml, suitable for a variety of applications from early screening to animal studies.

An add-on package is available for in-line dilution, allowing users to instantly reduce ethanol concentration during production. This further stabilizes LNPs right before collection.

NanoGenerator® Flex-M

Model	Flex-M	Flex-M Premium
Mixing Cartridge	CHP-MIX-4	CHP-MIX-4, CHP-MIX-PRO
Throughput	1 to 12 ml	1 to 200 ml
Max Flow Rate	5 mL/min w. MIX-4 mixer	5 mL/min w. MIX-4 mixer 24mL/min w. MIX-PRO mixer
Flow Rate Ratio (W:O)	1:1 to 5:1	1:1 to 10:1
In-line Dilution (optional)		0.5:1 to 2:1
Size Range		40 to 200 nm
Glass Chip Mixer		Compatible w. CHP-MIX-G1 & custom design mixer
PDI		0.05 to 0.2
Encapsulation Efficiency		85-95%

Batch-to-Batch Consistency

Batch#
(TFR 3ml/min, FRR W:O=3:1)

■ Z-Average(nm)   ● PDI

Tunable Total Flow Rate

Fixed Flow Rate Ratio (Aq:Lipid = 3:1)

■ Z-Average(nm)   ● PDI

Tunable Flow Rate Ratio

Flow Rate Ratio (Aq:Lipid)
Fxied TFR 5ml/min

■ Z-Average(nm)   ● PDI

Email: USSales@precigenome.com
Tel: +1-408-708-4602

Address: 2176 Ringwood Ave., San Jose, CA, USA
Visit us at www.precigenome.com/nanoparticle-synthesis

# NanoGenerator® Flex-M Premium Nanoparticle Synthesis System

NanoGenerator® Flex-M Premium

The NanoGenerator® Flex-M Premium can be upgraded from Flex-M with a hardware installation kit and software upgrade. It has a throughput range from 1 to 200 ml, suitable for a variety of applications from early screening to animal studies and pre-clinical applications.

An add-on package is available for in-line dilution, allowing users to instantly reduce ethanol concentration during production. This further stabilizes LNPs right before collection.

| Model	Flex-M	Flex-M Premium
Mixing Cartridge	CHP-MIX-4	CHP-MIX-4, CHP-MIX-PRO
Throughput	1 to 12 ml	1 to 200 ml
Max Flow Rate	5 mL/min w. MIX-4 mixer	5 mL/min w. MIX-4 mixer 24mL/min w. MIX-PRO mixer
Flow Rate Ratio (W:O)	1:1 to 5:1	1:1 to 10:1
In-line Dilution (optional)		0.5:1 to 2:1
Size Range		40 to 200 nm
Glass Chip Mixer		Compatible w. CHP-MIX-G1 & custom design mixer
PDI		0.05 to 0.2
Encapsulation Efficiency		85-95%

CHP-MIX-4 Vs. CHP-MIX-Pro
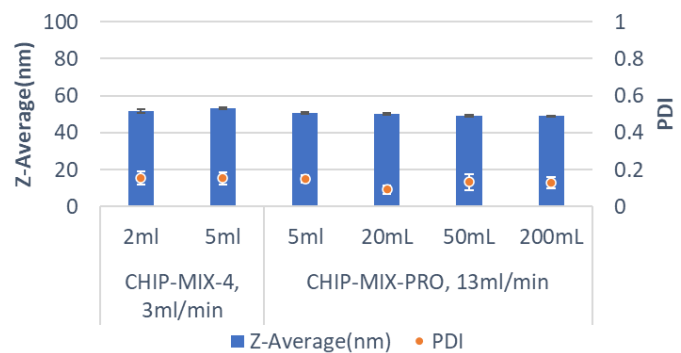
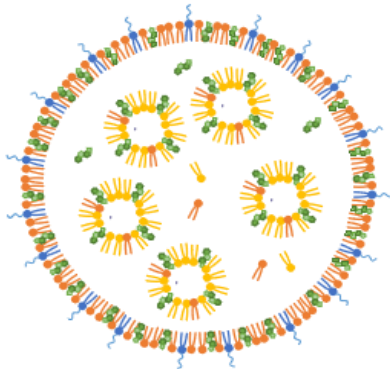## Tunable Total Flow Rate
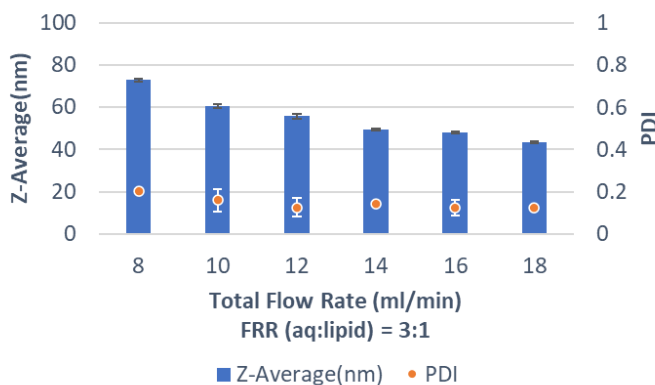
Tunable Flow Rate Ratio
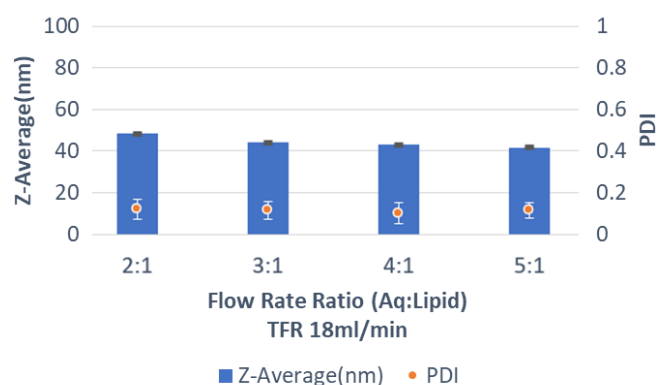
Case Study:

T Cell Transfection by mRNA Lipid Nanoparticles

Since the first FDA approval of chimeric antigen receptor (CAR) T cell therapy in 2017, T cell engineering has continued to be the hottest research field in immunotherapy and cell therapy. Current CAR T cell engineering methods use viral transductions, which induce permanent CAR expression and have potential safety concerns. To overcome these concerns, researchers are highly interested in non-viral gene delivery methods.

Recently, CAR mRNA LNPs in T cell engineering have been widely studied. The transient transduction of mRNA LNPs make them safer than viral vectors. With PreciGenome's NanoGenerator® platform, customers can produce mRNA LNPs with well controlled size, high homogeneity and excellent encapsulation efficiency, all key factors for efficient T cell transfection.

The following data shows the size and PDI of GFP mRNA LNPs synthesized by NanoGenerator® Flex systems. The transfection efficiency to K562 and HepG2 cell lines and human primary T cells are presented in Figures 2 and 3.
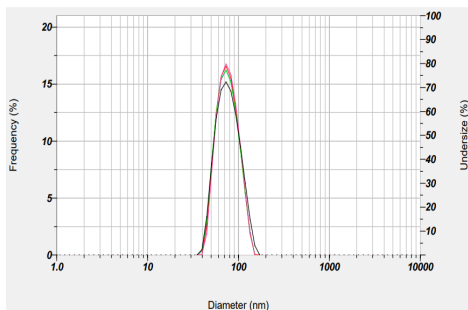

Figure 1. GFP-LNPs synthesized by PreciGenome's NanoGenerator® Flex-S. Average size is 67.3 nm. PDI is 0.106.
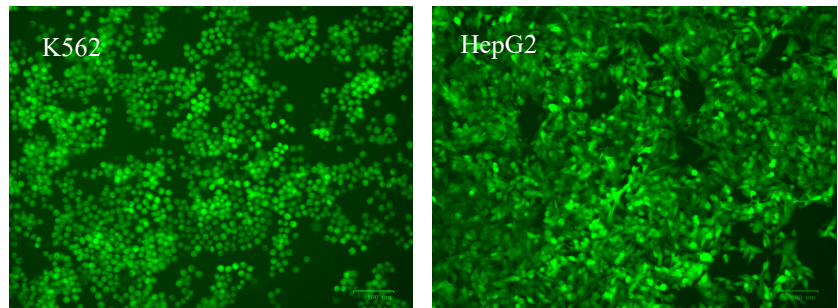

Figure 2. GFP expression in K562 (left) and HepG2 (right) cell lines 48 hours after treatment by GFP-LNPs synthesized by PreciGenome's NanoGenerator® Flex-S.

Figure 3. GFP(+) positive population of control (non-treated) and eGFP LNP treated primary T cells at 16, 40 and 64 hours.
Cells were stained (1:50) using BioLegend 7-AAD Viability Staining for 10 minutes.

Gating: First select for individual cells (excluding doublets). Then select for healthy cell population. Then select for viable cells by excluding cells positive for 7-AAD. Gate for FitC-A channel (GFP).
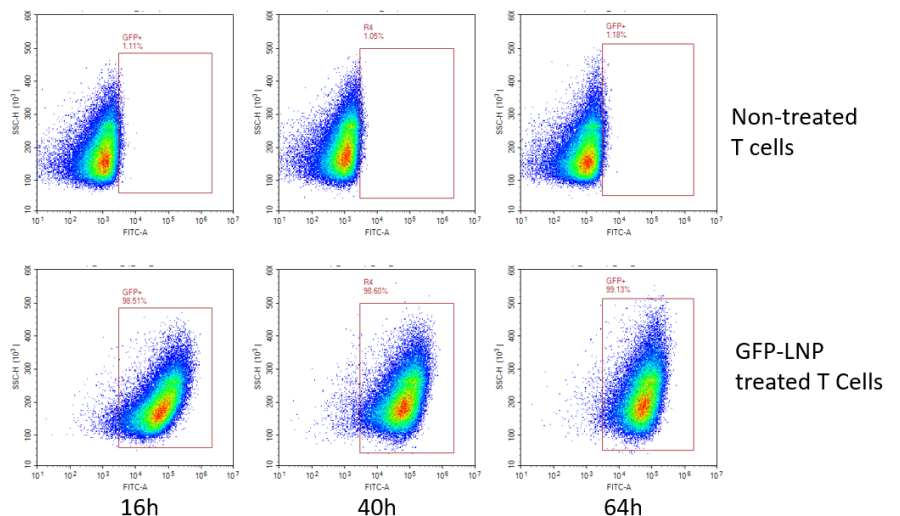
NanoGenerator® Max
Nanoparticle Synthesis System

With PreciGenome's microfluidic technology, customers can seamlessly transfer early discovery results (NanoGenerator® Flex, Pro) to the late stage production (NanoGenerator® Max).

The NanoGenerator® Max RUO version can be used for preclinical applications of LNP synthesis, while the NanoGenerator® Max GMP version is designed for clinical and commercial production.

Scalable RNA Encapsulation

High Encapsulation Efficiency
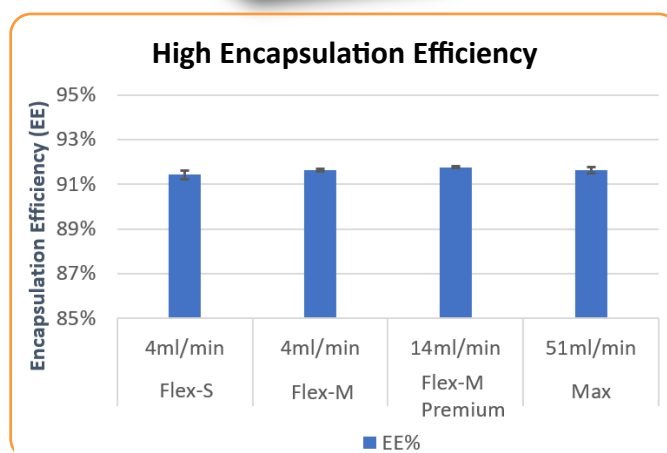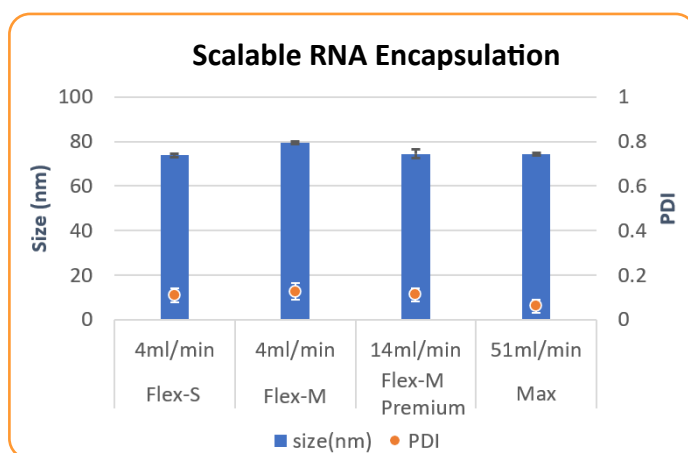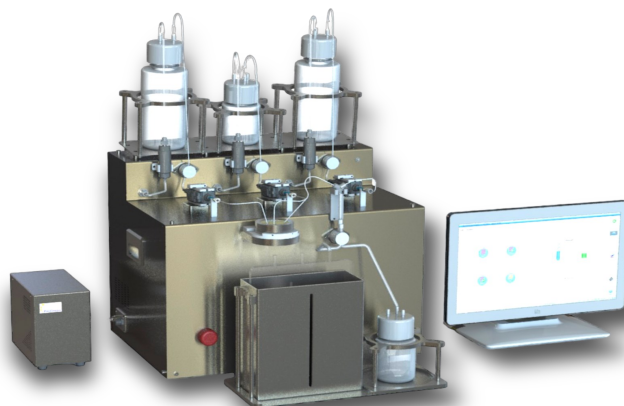
Model	NanoGenerator® MAX			
	RUO flow kit 4.8L/h	GMP flow kit 4.8L/h	RUO flow kit 40L/h	GMP flow kit 40L/h
cGMP compliance	N/A	Yes	N/A	Yes
Software (21 CFR Part 11 compliant)	Optional	Yes	Optional	Yes
Throughput	50ml – 1L		> 20L	
Max flow rate	4.8L/h		40L/h	
Flow rate ratio	1:1 – 9:1		1:1 – 5:1	
Inline dilution	1:1 – 5:1			
Size range	40 – 200 nm			
PDI	0.05 – 0.2			
Encapsulation efficiency	Up to 99%			
Payload	DNA, mRNA, siRNA, protein, small molecules, etc.			
Dimension (L × W × H)	620 × 380 × 430 mm			
Weight	50 Kg		65Kg	

The NanoGenerator® MAX GMP System is engineered to facilitate the production of genomic medicines for both clinical and commercial purposes.
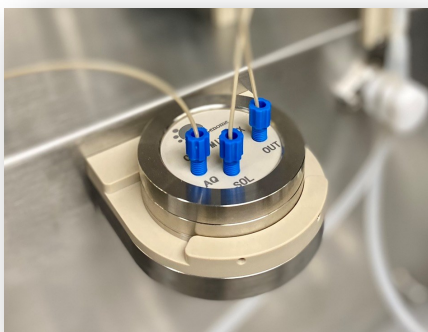
Regulatory support files for the single-use mixing flow pack are available including material traceability documentation .

PreciGenome has a proven history of delivering timely support to assist our customers in fulfilling their unique country- or region-specific regulatory requirements. The GMP System is manufactured under a Quality Management System.

cGMP Compliance Documentation

- Installation qualification, operational qualification, performance qualification
- Report of consumable extractables test
- Report of endotoxin test
- Report of RNase/DNase free test
- Report of sterility test
- Report of ethylene oxide residual test
- Report of consumable air tightness test
- Electromagnetic compatibility report
- Safety regulations report

Single-use mixing flow pack

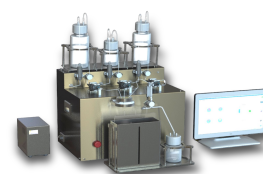The single-use mixing flow pack is fully documented to support regulatory and quality audits for cGMP production.

It reduces the risk of cross-contamination between batches and campaigns. It also enables multi-product manufacturing in GMP facilities.

Software (21 CFR Part 11 compliant) features

- Experimental parameter tuning
- Experimental recipe save/load
- Real-time pressure/flow rate chart
- Historic experimental parameter tracking
- Historic pressure/flow rate tracking
- Self-diagnostic system
- Real-time flow rate diagnostic system
- Warning system
- Manual & automatic emergency stop system
- User management
- Audit trail
- Zero flow calibration
- Flow sensor maintenance & re-calibration (service)

Email: USSales@precigenome.com
Tel: +1-408-708-4602

Address: 2176 Ringwood Ave., San Jose, CA, USA
Visit us at www.precigenome.com/nanoparticle-synthesis

BASIC FEATURES

BASIC FEATURES	Flex-S	Flex-S Plus	Flex-M	Flex-M Premium	MAX	MAX (40L/H)
Product Model Number	PG-SYN-FS	PG-SYN-SP	PG-SYN-FM	PG-SYN-FM	PG-SYN-G	PG-SYN-G
R&D Stage	Screening & Discovery	Screening & Discovery	Screening & Discovery	Discovery &Preclinical Studies	Preclinical Studies & Development	Clinical Development & Production
Throughput	0.1 to 2 ml	0.1 to 0.5 ml	1 to 12 ml	1 to 200 ml	50 ml to 1 L	>20L
Multiple Samples Per Run	✓	✓	✗	✗	✗	✗
Max Flow Rate	3 or 4 ml/min	3 or 4 ml/min	24 ml/min	24 ml/min	4.8 L/h	40L/h
Flow Rate Ratio	3:1	3:1	1:1 to 5:1	1:1 to 10:1	1:1 to 9:1	1:1 to 5:1
Tunable Flow Rate	Custom design	Custom design	✓		✓	✓
Intuitive & Easy To Use	✓	✓	✓		✓	✓
Inline Monitoring	Pressure	Pressure	Pressure & flow rate		Pressure & flow rate	Pressure & flow rate
Consumable Cost Per Run	$	$	$		$$	$$$

ADDITIONAL FEATURES (optional)

ADDITIONAL FEATURES	Flex-S	Flex-S Plus	Flex-M	Flex-M Premium	MAX	MAX (40L/H)
Inline Dilution	✗	✗	✓		✓	✓
Reagent Heating	✗	✗	✗		✓	✓
Custom Design/ OEM	✓	✓	✓		✓	✓
Qualification: IQ/ OQ	✓	✓	✓		✓	✓
21 CFR Part 11 Software	✓	✓	✓		✓	✓
Installation & Training	✓	✓	✓		✓	✓
Extended Warranty & PM Service	✓	✓	✓		✓	✓

LipidFlex™

## Flexible Lipid Nanoparticle Formulation

LipidFlex™ is a 3-component LNP formulation compatible with various cationic/ionizable lipids for nucleic acid encapsulation and cell transfection.
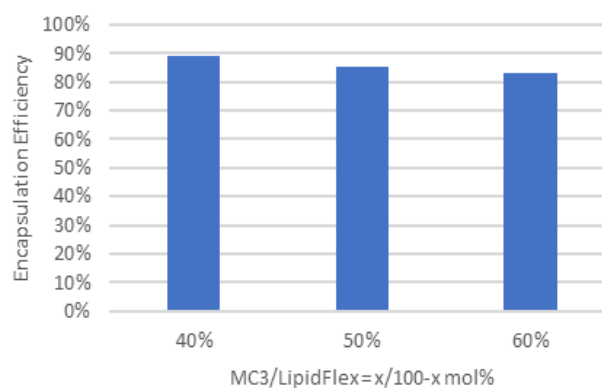
- Flexible cationic/ionizable lipid ratio
- Flexible with various N/P ratios
- High nucleic acid encapsulation efficiency
- High mammalian cell transfection rate

Model	LipidFlex™
Catalog #	PG-SYN-LF1ML
Components	Structural Lipid/Cholesterol/ Stabilizer
Product Size	1000 µl
Lipid Concentration	30 mM
Ionizable Lipid	NA

Size & PDI
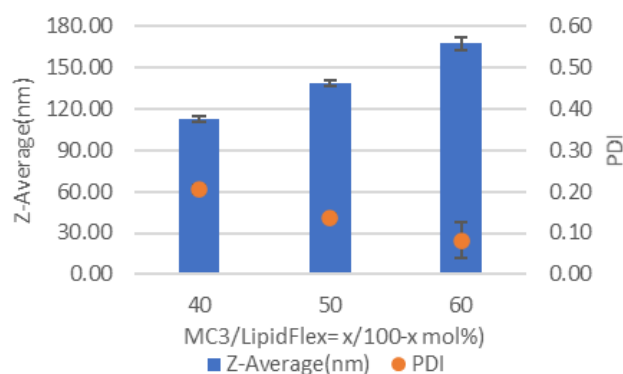
Encapsulation Efficiency

LipidFlex™ Experiment: HepG2 Cell Transfection Efficiency

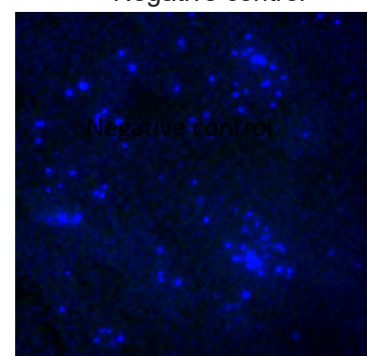| Sample	Positive control	Negative control
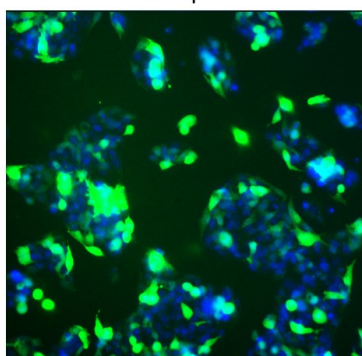
DNA LNP, PreciGenome NanoGenerator
SM102/PG-LipidFlex = 40/60 mol%
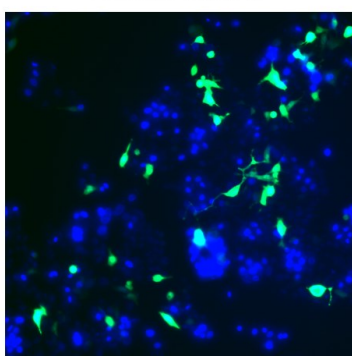
Lipofectamine™ 3000 (Thermo Fisher)

Non-treat

LipidFlex™ T Cell Kit

## High Efficient mRNA LNP Formulation for T Cell Transfection

The LipidFlex™ T cell kit is a highly efficient lipid formulation used to synthesize mRNA LNPs for primary human T cell gene delivery. Using the NanoGenerator® Flex-S and CHIP-MIX-4 cartridge, customers can efficiently prepare potent mRNA LNPs.

- Narrow size distribution of mRNA LNP

- High transfection efficiency

- High protein expression level

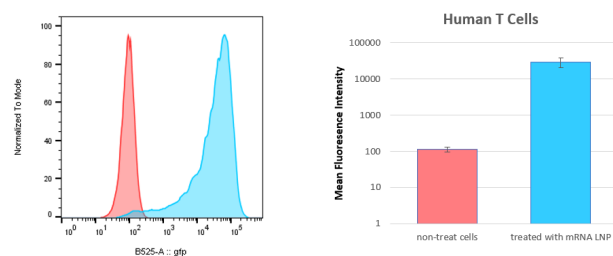- High cell viability

- Time efficient synthesis process

Component	Size	Storage
LipidFlex T Lipid mix	125 µl	-80°C
Formulation Buffer 1 (10x)	50 µl	4 to 8°C
Formulation Buffer 2	1 ml	4 to 8°C

High Human T Cell Transfection Efficiency
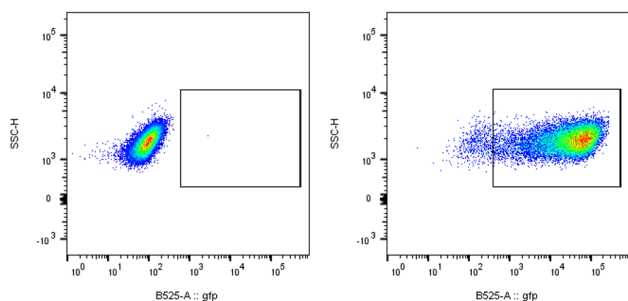
* 24 hours post-treatment Human T cells (eGFP mRNA from Trilink)
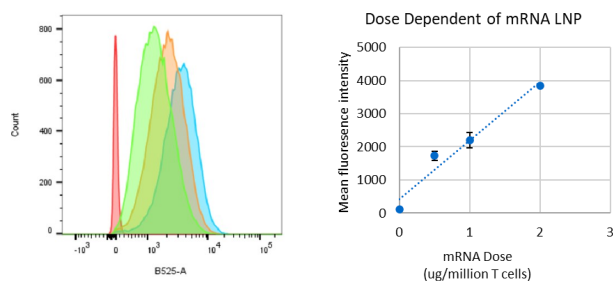
High Protein Expression Level

Non-treated cells   Treated with eGFP mRNA LNP
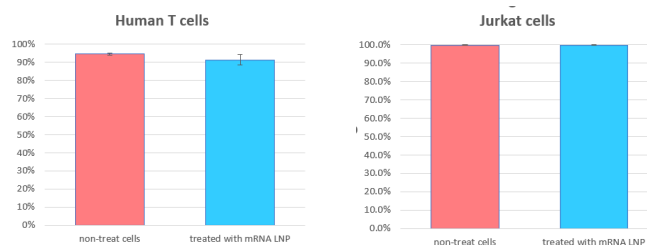
* 24 hours post-treatment Human T cells (eGFP mRNA from Trilink)

mRNA LNP Dose Dependence

* 24 hours post-treatment Jurkat cells (eGFP mRNA from ProMab)

High Cell Viability

* 24 hours post-treatment Human T cells and Jurkat cells

Email: USSales@precigenome.com
Tel: +1-408-708-4602

Address: 2176 Ringwood Ave., San Jose, CA, USA
Visit us at www.precigenome.com/nanoparticle-synthesis

PLGA Nanoparticle Synthesis with NanoGenerator®

The NanoGenerator® platform can be used for the synthesis of PLGA nanoparticles in addition to LNPs.

For PLGA nanoparticle synthesis, successful batch-to-batch consistency is empowered by the advanced microfluidic technology used in the CHP-MIX-4 (Flex-S) and the CHP-MIX-3 (Flex-M and PRO). Like with LNPs, this consistency applies across multiple throughput ranges, ensuring scalable results from 0.1 to 200 ml.

PLGA nanoparticle size tuning is controlled by formulation parameters, total flow rate and flow rate ratio. Supported flow rate conditions differ from standard LNP settings.
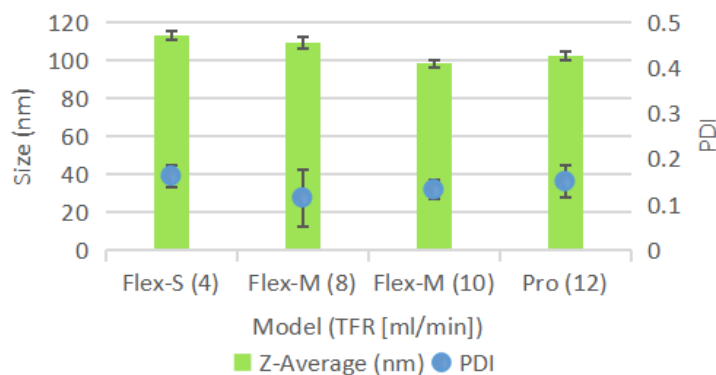

Batch-to-Batch Consistency (Flex-M)
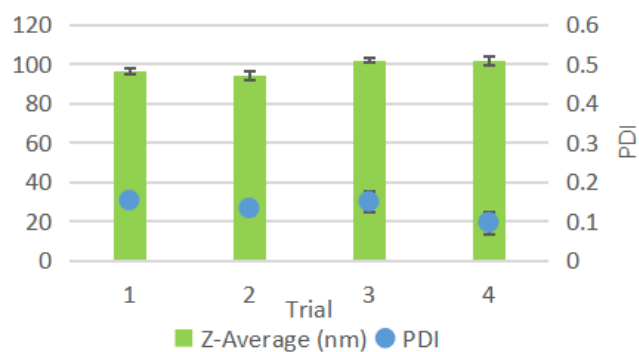
Batch-to-Batch Consistency (Flex-S)
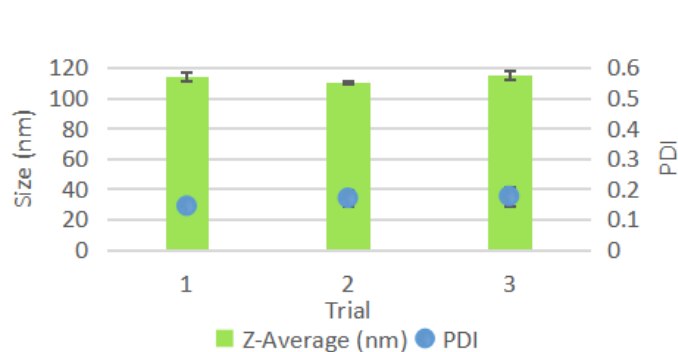
NanoGenerator™ Model Comparison

Customer Service

- **Formulation Design (LNP, Liposome, PLGA)**
  Customize nanoparticle design based on our clients' needs by adjusting lipid composition, vesicle size, surface charge, etc.
- **Payload Encapsulation**
  Customize protocols to encapsulate drugs into LNPs or PLGA NPs with high encapsulation efficiency.
- **Gene Delivery Study**
  In vitro cell study
  In vivo small animal study
- **Analysis and Characterization**
  Run comprehensive analysis assays for liposomes before and after encapsulation. Includes visual appearance, size distribution, stability, entrapment efficiency, encapsulation efficiency analysis, in vitro release profile analysis, release rate, etc.

Notes

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Notes

Some of Our Customers

![PreciGenome]

PreciGenome is located in the heart of Silicon Valley, San Jose, California, USA. We have been focusing on developing nanoparticle synthesis systems and solutions for our customers. Our technology enables nanoparticle synthesis with high quality and reliable performance for lipid nanoparticles, liposomes, PLGA, etc.

HEADQUARTER

PreciGenome LLC

Tel: 1-408-708-4602
Email: USSales@precigenome.com
Address: 2176 Ringwood Ave. San Jose, California, USA

TAIWAN DISTRIBUTOR

Ding Fong Scientific Co., Ltd

Tel: 886-4-23595717

Email: ding-fong@ding-fong.com.tw

CHINA DISTRIBUTOR

Suzhou Rainsure Biotech Co. Ltd

Tel: 86-512-67503398
Email: info@rainsurebio.com

KOREA DISTRIBUTOR

InSung Chroma-Tech Co., Ltd.

Tel: 82-226441991

Email: KRSales@precigenome.com

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