Define a Process for Your Unique mRNA: An Interactive Guide to pDNA, mRNA & LNP

Find out more: www.sartorius.com/mrna
Did You Know?

- pDNA is usually produced in *E. Coli* (intracellular) and can reach 1–2 g/L.
- pDNA are produced in 3 isoforms: supercoiled (sc), open circular (oc) and linear. Their ratio is process and plasmid dependant.
- pDNA size vary from 4 to 15 Kb.

Meet the Challenges

- Large plasmids are difficult to separate.
- Impurities to be removed are difficult to separate (hcDNA, RNA, endotoxins).

The pDNA Production Workflow

A linearized Plasmid DNA (pDNA), coding for the gene of interest, is typically used as a template for mRNA production during the In Vitro Transcription (IVT). A quality supply of pDNA is essential, as this critical raw material can impact IVT yield and mRNA quality.

Production Workflow

1. **Fermentation**
2. **Cell Harvest**
3. **Alkaline Lysis**
4. **Clarification**
5. **UF | DF**
6. **Capture and Polish**
7. **UF | DF**
8. **Sterile Filtration**

Optimize Your Process
Master. Purify. Scale-up.

Overcome Challenges With Solutions That Help You Optimize for a Next-Gen pDNA Production Strategy.

**Analytical Solution to Get Process Insights**

**MODDE®**
Design effective experiments to increase productivity, yield and quality.

**SIMCA®**
With an advanced data analytics solution you can model complex systems and gain deep process understanding.

**PATfix® HPLC**
Along with CIMac™ pDNA to separate sc | oc | linear.

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**Hollow fiber technology** is best suited for high content of processing suspended solids and large molecules. **Monolith technology** used in CIMmultus® and CIMac™ columns for chromatography shows low shear and works even for large plasmids.

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**1 TFF: Hollow Fiber Modules 750 KD – 0.1 µm**
**2 TFF: Hollow Fiber Modules 500 – 750 KD**
**3 TFF: Hollow Fiber Modules 100 – 300 KD**
**4 CIMmultus® HiP² Plasmid Process Pack** include CIMmultus® columns and process instructions to get pure sc pDNA.
**5 Sartopore® 2 XLG** suitable for large molecule containing a 0.8 µm prefiltre. Use SartoScale 25 (4.5 cm²) for process development.
The mRNA Production Workflow

mRNA is synthesized in a few hours, during an enzymatic reaction called In Vitro Transcription (IVT) using a linearized pDNA. Downstream steps are implemented to purify the mRNA and remove the IVT reagents (templates, enzymes...) and contaminants created such as double-stranded RNA.

Did You Know?
- High mRNA content vary depending on applications (15 mg for therapies vs 30 µg for COVID vaccine)
- saRNA, circRNA are also a new type of RNA
- mRNA construct have various structure and length (500 – 12,000 nt)
- mRNA is a large molecule (up to 30 times size of a mAb)

Meet the Challenges
- RNA is sensitive to hydrolysis, oxydation and RNase
- Impurity removal (truncated RNA, dsRNA, pDNA, enzymes...)
- No platform yet as there is a large variation in RNA construct
Master. Purify. Scale-up.

Overcome Challenges With Solutions That Help You Optimize for a Next-Gen mRNA Production Strategy.

1. **CIMmultus® C4-HLD** to purify the linearized plasmid.
2. **Ambr® 15 and 250**: multiple bioreactors for parallel IVT reaction to fasten process development.
3. **Hydrosart® SU TFF** cassette is a highly hydrophilic membrane for low mRNA absorption.

*Monolith technology* used in CIMmultus® and CIMac™ columns for chromatography shows low shear and works even for large plasmids.

**Hydrosart® SU TFF** cassette is a highly hydrophilic membrane for low mRNA absorption.

*Production Workflow*

- **Sartocon® Slice 50 Hydrosart** (50 cm²) used with Ambr® Crossflow for process development.
- **CIMmultus® Toolbox** OligoT (affinity), PrimaS® (AEX), C4HLD (HIC) SDVB (reverse phase) to fit any process | RNA construct. Use 96 well plate for process development.
- **Sartopore® 2 XLG** suitable for large molecule with a 0.8 µm prefiter. Use SartoScale 25 (4.5 cm²) for process development.
- **Celsius® family** for a stable storage and shipment to LNP encapsulation facility. Starting from 30 mL bag for your process development.

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**Optimize Your Process**

**Analytical Solution to Get Process Insights**

**MODDE®**
Design effective experiments to increase productivity, yield and quality.

**SIMCA®**
With an advanced data analytics solution you can model complex systems and gain deep process understanding.

**PATfix® HPLC**
Along with CIMac™, pDNA can separate linear from other isoforms. Along with CIMac™ PrimaS®. Oligo dT or SDVB you will be able to quantify mRNA and IVT components, verify mRNA size, separate dsRNA | mRNA.

**Sartorius**
The LNP Production Workflow

mRNA is encapsulated into Lipo-NanoParticles (LNPs) to protect it against degradation and facilitate entrance into the patient’s cells. The LNPs are purified before form and finish.

- **Lipids composition of LNP may vary to optimize cellular uptake, endosomal escape, mRNA payload…**
- **LNP size varies from 50 – 150 nm**

Meet the Challenges

- **Aggregation and Stability**
- **Control of lipid type, source and quality**

Did You Know?

- Lipids composition of LNP may vary to optimize cellular uptake, endosomal escape, mRNA payload...
- LNP size varies from 50 – 150 nm

Ready for Larger Production?

Build your mRNA facility of the future! Conceptual design services can take you from idea to concept design in 10 weeks.

Consult Our Experts
Master. Purify. Scale-up.

Overcome Challenges With Solutions That Help You Optimize for a Next-Gen LNP Production Strategy.

MODDE®
Design effective experiments to increase productivity, yield and quality.

SIMCA®
With an advanced data analytics solution you can model complex systems and gain deep process understanding.

Hydrosart® SU TFF cassette is a highly hydrophilic membrane for low mRNA absorption.

1 Hydrosart® cut off of 30, 100 and 300 KDa depending on your LNP size. Sartocon® Slice 50 Hydrosart® (50 cm²) used with Ambr® Crossflow for your process development.

Or Hollow Fiber Modules cut off of 100 or 300 KDa for very gentle ultrafiltration.

2 Sartopore® 2 XLG is suitable for large molecule with a 0.8 µm prefiler. Use SartoScale 25 (4.5 cm²) for process development.

3 Celsius® family for a stable storage and shipment to the filling line. Starting from 30 mL bag for your process development.