# PROTOCOL

# LipidBrick® IM21.7c
# Cationic lipid for LNP formulation

## DESCRIPTION

As an innovator in the field of nucleic acid delivery, Polyplus® has developed a new range of cationic lipids, named LipidBrick®, dedicated to the formulation lipid nanoparticles (LNPs). These "active lipids" protect nucleic acids such as mRNA, siRNA or DNA and deliver them to the cells. Based on an imidazolium polar head, LipidBrick® IM21.7c broadens the current applications spectrum in terms of potency and targeting by adding positive charges to LNPs, as the electric charge of LNPs is known to impact their biodistribution and nucleic acid expression.

| | |
|---|---|
| **Name** | LipidBrick® IM21.7c<br>3-butyl-1-(2,6-dimethyl-14-octadecyldotriacontan-9-yl)-1H-imidazol-3-ium chloride |
| **Type** | Cationic lipid |
| **Linear Formula** | $C_{59}H_{117}ClN_2$ |
| **Molecular weight** | 890.03 g/mol |
| **CAS number** | 2416939-42-7 |

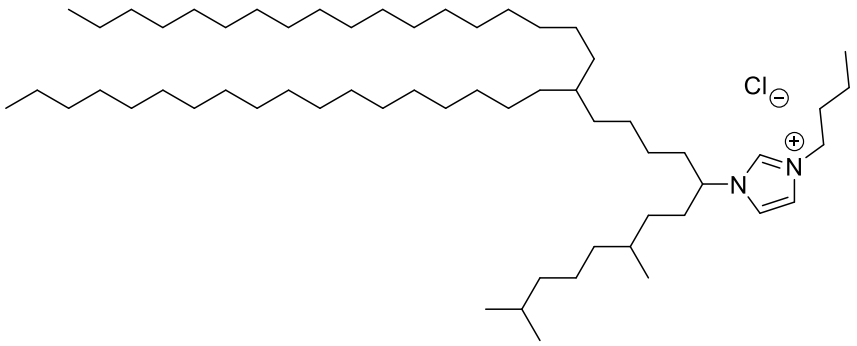


**Figure 1: LipidBrick® IM21.7c**



Polyplus

# 1. LNP formulation

Lipid nanoparticles (LNPs) are the non-viral delivery system of choice for DNA and RNA therapeutics. Their lipidic structure encapsulates and protects nucleotides from degradation, prolongs their circulation in the body and facilitates their uptake into cells using different routes of administration (systemic or local injections). These properties make LNPs an attractive option for the delivery of drug products.

LNPs are composed of 3 types of molecules:

- Active lipids (cationic and/or ionizable lipids): positively charged lipids interact with negatively charged nucleic acids and mediate disruption of the endosome to allow release of nucleic acid into the cytoplasm,
- Structural lipids (phospholipids): ensure rigidity and stability of LNPs,
- Modulators:
  - PEG-lipids: provide steric stabilization and prolong blood circulation,
  - Cholesterol: increases the fluidity and stability of the LNP membrane and promotes membrane fusion with the cells.

The manufacturing process of LNPs is made using microfluidic systems that will homogenize the nucleic acids (mRNA, DNA, siRNA, or other types) and the lipid mixture in a laminar flow.
First, cationic or ionizable lipids interact with and encapsulate nucleic acids. Intermediate nanostructures will make hydrophobic lipid tails available to interact with structural and modulatory lipids. The LNP structure will then be finalized by the formation of functional vesicles capable of protecting and delivering nucleic acids (Fig. 2).

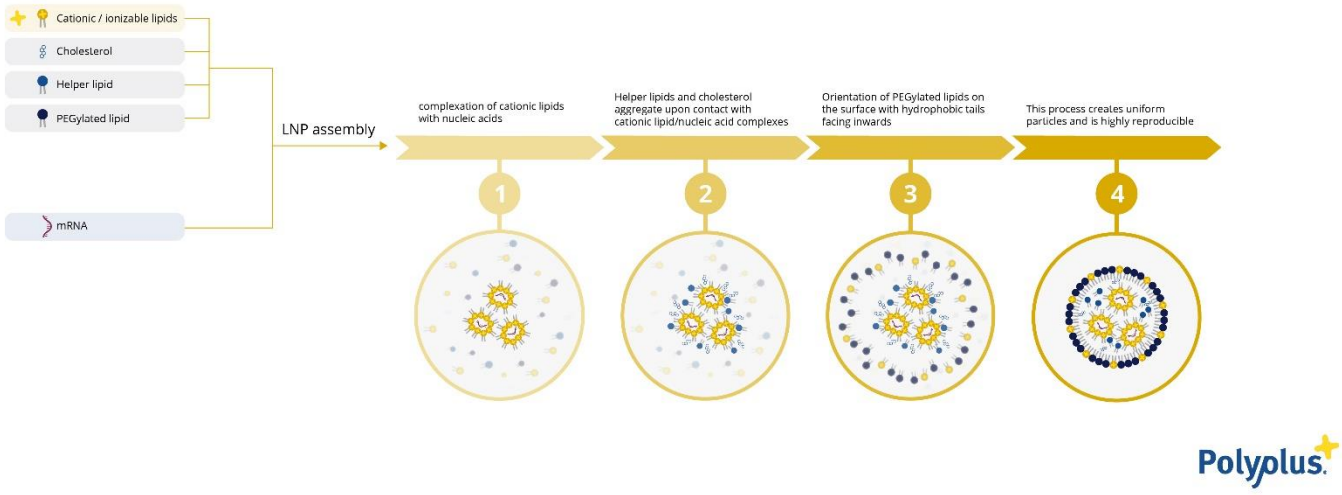


**Figure 2: LNPs formulation process using microfluidic systems**

## 1.1. Suggested equipment and raw material

**Table 1. Third-party equipment (not supplied) required for the formulation of LNPs with LipidBrick®**

| Description | Example of equipment | Supplier |
|---|---|---|
| **Microfluidic equipment** | NanoAssemblr® Ignite™ | Precision NanoSystems |
| **Particle size analyzer** | Zetasizer Nano-ZS | Malvern Panalytical |
| **Microplate reader** | FLUOstar® Omega | BMG Labtech |
| **Bench Top Centrifuge** | - | - |
| **Vortex Mixer** | - | - |

**Table 2. Third-party consumables (not supplied) required for the formulation of LNPs with LipidBrick®**

| Description | Example of consumable & supplier |
|---|---|
| **Cartridge** | NanoAssemblr® Ignite™ NxGen cartridges, Precision NanoSystems |
| **Cuvettes for particle size analyzer** | 1.5-3.0 mL UV Cuvettes, VWR |
| **Centrifugal filter concentrator** | • Vivaspin® Turbo 4, 10kDa, Sartorius<br>• Amicon® Ultra-15 centrifugal filter units, 10kDa, Millipore |
| **Filter** | PES filter 0.45 µm 17 mm |
| **Syringes** | BD Syringes 1 mL, BD Plastipak™<br>BD Syringes 10 mL, BD Plastipak™ |
| **Needles** | Needles 20G*1'', Terumo™<br>Needles 21G*1 ½'', Terumo™ |

**Table 3. Third-party reagents (not supplied) required for the formulation of LNPs with LipidBrick®**

| Example of reagent | Supplier |
|---|---|
| CleanCap® mRNA | TriLink |
| Quant-iT™ RiboGreen™ RNA Assay Kit | ThermoFisher Scientific |

## 1.2. Preparation of LNPs with LipidBrick® IM21.7c

1. All lipids are dissolved in ethanol and the final molar ratios are established as shown in Table 4. For example, we recommend solubilizing LipidBrick® IM21.7c powder in EtOH at a concentration of 100 mM. Solubilization can be carried out at up to 37°C in an ultrasonic bath for 30 minutes, followed by a rapid vortexing. Once solubilized, LipidBrick® IM21.7c can be stored at 4°C for long-term storage.
2. mRNA is diluted in 10 mM sodium acetate pH 4 buffer.
3. mRNA-containing LNPs are prepared by combining the lipid and mRNA solutions in a NanoAssemblr™ microfluidic cartridge at a flow rate of 10 mL/min and volumetric ratio of 3:1.
4. Ethanol is removed using Amicon® or Vivaspin® centrifugal filter units (10 kDa cutoff). LNPs are filtrated through a PES filter (0.45 µm 17 mm) and diluted in PBS.

**Table 4. Optimized LNP formulation**

| Lipids | Stock concentration (mM) | Molar Ratio |
|---|---|---|
| Cationic lipid | LipidBrick® IM21.7c (100 mM) | 10 – 40 % |
| Ionizable lipid | DODMA (50 mM) | 20 – 50 % |
| Helper lipid | DPyPE (30 mM) | 10 % |
| Cholesterol lipid | Cholesterol (50 mM) | 15 – 30 % |
| PEG lipid | DSG-$PEG_{2k}$ (10 mM) | 1.5 – 5 % |

## 1.3. Successfully tested lipids

Various LNP formulations with LipidBrick® IM21.7c were successfully tested in-house with different active lipids, structural lipids and modulators (Table 5).

**Table 5. List of lipids successfully tested with LipidBrick® IM21.7c**

| Lipids | Examples |
|---|---|
| Cationic lipid | LipidBrick® IM2.7c |
| Ionizable lipid | DODMA, DLin-MC3-DMA, SM-102, ALC-0315 |
| Helper lipid | DPyPE, DOPE, DSPC |
| Cholesterol lipid | Cholesterol |
| PEG lipid | DSG-$PEG_{2k}$, DMG-$PEG_{2k}$ |

## 1.4. *In vitro* evaluation of mRNA-LNPs

For optimal mRNA transfection conditions, we recommend using cells which are 60 to 80% confluent at the time of transfection. Typically, for experiments in 24-well plate, between 40 000 to 100 000 adherent cells are seeded per well in 0.5 mL of cell growth medium 24 h prior to transfection (Table 6).

1. Add 10 µL (or 5 µL, as shown in Table 6) of mRNA-LNPs (mRNA 50 ng/µL) per well dropwise onto the cells in growth medium and/or additives and distribute evenly.
2. Gently rock the plate back and forth and from side to side.
3. Analyze gene expression 24 - 48 h after the transfection.

**Table 6. mRNA-LNPs transfection guidelines conditions for various cell**

| Cell type | Cells | Number of cells to seed per well of a 24-well plate | Amount of mRNA (ng) | Volume of LNPs (50 ng/µL) |
|---|---|---|---|---|
| Epithelial | Caco-2 | 40,000 | 500 | 10 µL |
| | A549 | 60,000 | 500 | 10 µL |
| | HeLa | 50,000 | 250 | 5 µL |
| | HEK-293 | 50,000 | 250 | 5 µL |
| Hepatocyte | HepG2 | 100,000 | 500 | 10 µL |

# 2. Recommendations

Based on our proof-of-concept study, we recommend using the following positive control for the development of your LNPs with LipidBrick® IM21.7c:

- *in vivo*-jetRNA®+: a ready-to-use liposome-based transfection reagent containing LipidBrick® IM21.7c as cationic lipid (available in our Polyplus® product portfolio),
- The LNP formulation described in the Table 7.

**Table 7. LNP formulation recommended as positive control**

| Lipids | Molar Ratio mM |
|---|---|
| LipidBrick® IM21.7c | 4 mM |
| DODMA | 3 mM |
| DPyPE | 1 mM |
| Cholesterol | 1.85 mM |
| DSG-$PEG_{2k}$ | 0.15 mM |

# 3. Troubleshooting

**Table 8. Troubleshooting for LNP formulation and *in vitro* transfection**

| Observations | Actions |
|---|---|
| **Filtration issues** | • Use Amicon® instead of Vivaspin® filtration units or *vice versa* |
| **Cellular toxicity** | • Replace medium 4 h after transfection.<br>• Analyze transfection at an earlier time point (e.g. at 24 h instead of 48 h).<br>• Decrease the amount of mRNA added per well. |
| **Scale-up concerns** | • Contact us online for tips and advice: support@polyplus-transfection.com |

# 4. Product information

## 4.1. Ordering information

| Product | Part N° | Quantity |
|---|---|---|
| **LipidBrick® IM21.7c** | **101000172** | 250 mg |
| **LipidBrick® IM21.7c** | **101000173** | 1 g |

## 4.2. Reagent use and limitations

For research use only. Not for use in humans.

## 4.3. Quality control

Each batch of LipidBrick® is tested in-house to assess the identity of the product. The results are recorded in Certificates of Analysis, available online in your customer area: https://myaccount.polyplus-transfection.com/wp-login.php.

## 4.4. Formulation and storage

LipidBrick® should be stored at -20°C upon arrival to ensure long-term stability.

Polyplus-transfection® has been awarded ISO 9001 Quality Management System Certification since 2002, which ensures that the company has established reliable and effective processes for manufacturing, quality control, distribution, and customer support.

## 4.5. Trademarks

Polyplus® and LipidBrick® are registered trademarks of Polyplus-transfection S.A.

How to cite us: “LipidBrick® IM21.7c (Polyplus-transfection S.A, Illkirch, France)”.

## 4.6. Contact information

**Do you have any technical question regarding your product?**

- Website: www.polyplus-transfection.com
- Email: support@polyplus-transfection.com
- Phone: +33 3 90 40 61 87

Contact our friendly Scientific Support team which is composed of highly educated scientists, PhDs and Engineers, with extensive hands-on experience in cell culture and transfection. The Scientific Support team is dedicated to help our customers reach their goals by offering various services such as: protocol optimization, personalized transfection conditions, tailored protocols, etc.

Please note that the Polyplus-transfection® support is available by phone from 9:00 am to 5:00 pm CEST.

**For any administrative question, feel free to contact our administration sales team:**

- Reception Phone: +33 3 90 40 61 80
- Fax: +33 3 90 40 61 81
- Addresses:

| Polyplus® locations | Addresses |
|---|---|
| Headquarter<br>Transfection reagent manufacturing site | 75, rue Marguerite Perey<br>67400 Illkirch<br>France |
| Plasmid design site | 80 Rue du Dr Yersin<br>59120 Loos<br>France |
| US Sales Office | 1251 Ave of the Americas<br>34th fl.<br>New-York - NY 10020<br>United States |
| China Sales Office | Room 1506, Tower B, Sunyoung Center<br>No. 28 Xuanhua Road<br>Changning District, Shanghai<br>China |