PEG2000-C-DMG

Cat. No.: HY-145411

Molecular Formula: $C_{126}H_{249}NO_{52}$

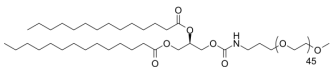
Molecular Weight: 2610.29

Target: Liposome

Pathway: Metabolic Enzyme/Protease

Storage: -20°C, stored under nitrogen

* In solvent : -80°C, 6 months; -20°C, 1 month (stored under nitrogen)



SOLVENT & SOLUBILITY

In Vitro

DMSO : 100 mg/mL (38.31 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	0.3831 mL	1.9155 mL	3.8310 mL
	5 mM	0.0766 mL	0.3831 mL	0.7662 mL
	10 mM	0.0383 mL	0.1915 mL	0.3831 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (0.96 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (0.96 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (0.96 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

PEG2000-C-DMG, a pegylated lipid, can be used for the preparation of Onpattro. Onpattro, a hepatically directed investigational RNAi therapeutic agent, harnesses this process to reduce the production of mutant and wild-type transthyretin by targeting the 3′ untranslated region of transthyretin mRNA[1][2].

In Vitro

Preparation of MC3 Lipid Nanoparticles

Here we provide lipid molar ratios for LNPs in FDA-approved Patisiran (a siRNA targets the transthyretin (TTR) mRNA). The molar ratio of lipids in this formulation is D-Lin-MC3-DMA : DSPC : Cholesterol : PEG2000-C-DMG = 50 : 10 : 38.5 : 1.5[1], and

RNA to lipid weight ratio is 0.05 (wt/wt).

A. Lipid Mixture Preparation

1. Dissolve lipids in ethanol and prepare 10 mg/m stock solutions. The lipid stock solutions can be stored at −20°C for later use.

Note 1: The ionizable lipid is usually a liquid. Due to the viscosity, it should always be weighed rather than relying on the autopipette volume.

Note 2: Cholesterol in solution should be kept warm (>37℃) to maintain fluidity. Transfer the cholesterol solution promptly to avoid cooling.

2. Prepare the lipid mixture solution as described. For each mL of lipid mixture add the following: 548 µL of 10mg/mL D-Lin-MC3-DMA (HY-112251), 254 µL of 10mg/mL Cholesterol (HY-N0322), 134 µL of 10mg/mL DSPC (HY-W040193), and 64 µL of PEG2000-C-DMG (HY-145411) [2]. Mix the solutions thoroµghly to achieve a clear solution. This mixture contains 10 mg of total lipid.

Note 3: The choice of lipids and ratios may be changed as desired and this will affect the LNP properties (size, polydispersity, and efficacy) and the amount of mRNA required.

B. siRNA Preparation

1. Prepare a 166.7 µg/mL siRNA solution with 100 mM pH 5 sodium acetate buffer.

Note 4: The lipid:siRNA weight ratio influences the encapsulation efficiency. Other weight ratios may be prepared as alternative formulations and should be adjusted accordingly by user.

C. Mixing

There are three commonly used methods to achieve rapid mixing of the solutions: the pipette mixing method, the vortex mixing method, and the microfluidic mixing method. All these mixing methods can be used for various applications.

It is important to note that pipette mixing method and vortex mixing method may yield more heterogeneous LNPs with lower encapsulation efficiencies and is prone to variability. Microfluidic devices enable rapid mixing in a highly controllable, reproducible manner that achieves homogeneous LNPs and high encapsulation efficiency. Within these devices, the ethanolic lipid mixture and aqueous solution are rapidly combined in individual streams. LNPs are formed as the two streams mix and are then collected into a single collection tube.

1. Pipette Mixing Method:

1.1. Pipette 3 mL of the siRNA solution and quickly add it into 1 mL of the lipid mixture solution (A 1:3 ratio of ethanolic lipid mixture to aqueous buffer is generally used.) Pipette up and down rapidly for 20–30 seconds.

1.2. Incubate the resulting solution at room temperature for up to 15 minutes.

1.3. After mixing, the LNPs were dialyzed against PBS (pH 7.4) for 2 h, sterile filtered using 0.2 µm filters, and stored at 4°C.

2. Vortex Mixing Method:

1.1. Vortex 3 mL of siRNA solution at a moderate speed on the vortex mixer. Then, Quickly add 1 mL of the lipid mixture solution into the vortexing solution (A 1:3 ratio of ethanolic lipid mixture to aqueous buffer is generally used.). Continue vortexing the resulting dispersion for another 20–30 seconds.

1.2. Incubate the resulting solution at room temperature for up to 15 minutes.

1.3. After mixing, the LNPs were dialyzed against PBS (pH 7.4) for 2 h, sterile filtered using 0.2 µm filters, and stored at 4°C.

3. Microfluidic Mixing Method:

1.1 The 3 mL of siRNA buffer solution and 1 mL of the lipid mixture solution were mixed at a total flow rate of 12 mL/min (A

1:3 ratio of ethanolic lipid mixture to aqueous buffer is generally used.) in a microfluidic device.

Note 5: Parameters such as the flow rate ratio and total flow rate can be altered to fine-tune LNPs.

1.2. After mixing, the LNPs were dialyzed against PBS (pH 7.4) for 2 h, sterile filtered using 0.2 μm filters, and stored at 4°C.

Reference

1. Curr Issues Mol Biol. 2022 Oct 19;44(10):5013-5027.

2. Curr Protoc. 2023;3(9):e898.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Garber K. Alnylam launches era of RNAi drugs. Nat Biotechnol. 2018;36(9):777-778.

[2]. Adams D, Gonzalez-Duarte A, O'Riordan WD, et al. Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. N Engl J Med. 2018;379(1):11-21.

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