DMG-PEG 2000

Cat. No.: HY-112764

CAS No.: 160743-62-4

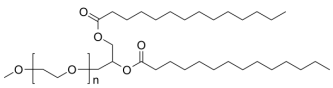
Molecular Formula: $(C_2H_4O)nC_{32}H_{62}O_5$

Molecular Weight: 2526

Target: Liposome

Pathway: Metabolic Enzyme/Protease

Storage: -20°C, sealed storage, away from moisture and light
* In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture and light)



SOLVENT & SOLUBILITY

In Vitro

DMSO : 125 mg/mL (49.49 mM; ultrasonic and warming and heat to 60°C)
Ethanol : 100 mg/mL (39.59 mM; Need ultrasonic)
H_2O : 16.67 mg/mL (6.60 mM; Need ultrasonic)

Preparing Stock Solutions	Mass / Solvent / Concentration	1 mg	5 mg	10 mg
	1 mM	0.3959 mL	1.9794 mL	3.9588 mL
	5 mM	0.0792 mL	0.3959 mL	0.7918 mL
	10 mM	0.0396 mL	0.1979 mL	0.3959 mL

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 90% saline
   Solubility: ≥ 10 mg/mL (3.96 mM); Clear solution
2. Add each solvent one by one: 5% DMSO >> 95% saline
   Solubility: ≥ 5 mg/mL (1.98 mM); Clear solution
3. Add each solvent one by one: 10% EtOH >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (0.99 mM); Clear solution
4. Add each solvent one by one: 10% EtOH >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (0.99 mM); Clear solution
5. Add each solvent one by one: 10% EtOH >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (0.99 mM); Clear solution
6. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.08 mg/mL (0.82 mM); Clear solution
7. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (0.82 mM); Clear solution
8. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (0.82 mM); Clear solution

BIOLOGICAL ACTIVITY

Description	DMG-PEG 2000 is used for the preparation of liposome for siRNA delivery with improved transfection efficiency in vitro. DMG-PEG 2000 is also used for the lipid nanoparticle for an oral plasmid DNA delivery approach in vivo through a facile surface modification to improve the mucus permeability and delivery efficiency of the nanoparticles[1].
In Vitro	Preparation of Lipid Nanoparticles Here we provide lipid molar ratios for LNPs in FDA-approved mRNA-1273 (a COVID-19 mRNA vaccine). The molar ratio of lipids in this formulation is SM-102 : DSPC : Cholesterol : DMG-PEG 2000 = 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: 572 µL of 10mg/mL SM-102 (HY-134541), 240 µL of 10mg/mL cholesterol (HY-N0322), 127 µL of 10mg/mL DSPC (HY-W040193), and 61 µL of DMG-PEG 2000 (HY-112764). 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. mRNA Preparation 1. Prepare a 166.7 µg/mL mRNA solution with 100 mM pH 5 sodium acetate buffer. Note 4: The lipid:mRNA 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 from: 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 mRNA 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 mRNA 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 mRNA buffer solution and 1 mL of the lipid mixture solution were mixed at a total flow rate of 12 mL/min in a microfluidic device (A 1:3 ratio of ethanolic lipid mixture to aqueous buffer is generally used.). 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.
In Vivo	NP-3 (oral administration; 150 μg DNA per mouse; single dose) at 12, 24, and 36 h postadministration, luciferin substrate is intraperitoneally injected to verify its permeability. NP-3 group maintains high luciferase expression in the liver, lung, and intestine areas 12-24 h post-treatment.Additionally, NP-3 exhibits 1.5 times higher signal intensity than that of NP-1 or NP-2 group from 12 to 24 h postoral administration[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- J Biomed Sci. 2023 Jun 28;30(1):46.
- J Biomed Sci. 2022 Dec 22;29(1):108.
- J Biomed Sci. 2022 Jul 7;29(1):49.
- University of Toronto. 2023 Nov.

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REFERENCES

[1]. Tianqi Nie, et al. Surface Coating Approach to Overcome Mucosal Entrapment of DNA Nanoparticles for Oral Gene Delivery of Glucagon-like Peptide 1.ACS Appl Mater Interfaces. 2019 Aug 21;11(33):29593-29603.

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