上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

产品名称：ALC-0159

CAS 号： 1849616-42-7

产品货号：S89625

生物活性：

Description	ALC-0159 是一个聚乙二醇的脂质结合物，可用作疫苗赋形剂。
In Vitro	Preparation of Lipid Nanoparticles Here we provide lipid molar ratios for LNPs in FDA-approved BNT162b2 (a COVID-19 mRNA vaccine). The molar ratio of lipids in this formulation is ALC-0315 : DSPC : Cholesterol : ALC-0159 = 46.3 : 9.4 : 42.7 : 1.6, and RNA to lipid weight ratio is 0.05 (wt/wt)[1]. 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°C) 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: 560 µL of 10mg/mL ALC-0315 , 261 µL of 10mg/mL Cholesterol , 117 µL of 10mg/mL DSPC , and 62 µL of 10mg/mL ALC-0159 . 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: 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:

上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

	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.
Solvent&Solubility	**细胞实验:** DMSO 中的溶解度 ：100 mg/mL (超声助溶；吸湿的 DMSO 对产品的溶解度有显著影响，请使用新开封的 DMSO) Ethanol 中的溶解度 ：≥ 50 mg/mL * "≥" means soluble, but saturation unknown
	动物实验： 动物实验： 请根据您的实验动物和给药方式选择适当的溶解方案。 以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂： ——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶 方案 一 请依序添加每种溶剂： 10% DMSO → 40% PEG300 → 5% Tween-80 → 45% Saline Solubility: ≥ 2.5 mg/mL; 澄清溶液 此方案可获得 ≥ 2.5 mg/mL（饱和度未知）的澄清溶液。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；再向上述体系中加入 50 μL Tween-80，混合均匀；然后再继续加入 450 μL 生理盐水 定容至 1 mL。 生理盐水的配制：将 0.9 g 氯化钠，溶解于 ddH_2O 并定容至 100 mL，可以得到澄清透明的生理盐水溶液。 方案 二 请依序添加每种溶剂： 10% DMSO → 90% (20% SBE-β-CD in Saline) Solubility: ≥ 2.5 mg/mL; 澄清溶液 此方案可获得 ≥ 2.5 mg/mL（饱和度未知）的澄清溶液。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液 中，混合均匀。

上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

	2 g SBE-β-CD（磺丁基醚 β-环糊精）粉末定容于 10 mL 的生理盐水中，完全溶解至澄清透明。 方案 三 请依序添加每种溶剂： 10% DMSO → 90% Corn Oil Solubility: ≥ 2.5 mg/mL; 澄清溶液 此方案可获得 ≥ 2.5 mg/mL（饱和度未知）的澄清溶液，此方案实验周期在半个月以上的动物实验酌情使用。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
参考文献	[1]. S Moein Moghimi, et al. Allergic Reactions and Anaphylaxis to LNP-Based COVID-19 Vaccines. Mol Ther. 2021 Mar 3;29(3):898-900.