Gene / nucleic acid transfection reagent

COSMO BIO CO., LTD.
Inspiration for Life Science

LipoTrust™ *EX* Oligo *<in vivo>*

■ Contents

LipoTrust™ *EX* Oligo *<in vivo>*

■ Amount and Storage

Lyophilizate for 1ml use (containing 10μmol cationic lipid/ vial) Storage at 2-8 C

■ Description

LipoTrust™ *EX* Oligo *<in vivo>* is a proprietary cationic liposome formulation that facilitates highly efficient delivery of short oligonucleotides such as siRNA, antisense DNA or miRNA into organs or tissues. Customers can easily prepare liposomal suspension for highly effective transfection with direct mixing of short oligonucleotides solution into lyophilized powder.

■ How to apply for vivo use

Transfection Procedure with LipoTrust™ *EX* Oligo*<in vivo>* and administration

(1) Prepare 80 pmol/μl (1 mg/ml) oligonucleotide solutions[*1] with sterile and Nuclease free water.
(2) Open the cap of **LipoTrust™*EX* Oligo** for *in vivo* vial and pour 1 ml of above prepared oligonucleotide solution into the vial.
(3) Mix the liquid well by which liquid are allowed in and out from the vial at least 10 times using clean disposable pipette. After this procedure, the liquids will be kept for about 20 minutes at room temperature.
(4) Apply the mixed liquid to animals (80 μl/1 mouse is usually appropriate[*2]).

[*1] Less than 80 pmol/μl (1 mg/ml) oligo-nucleotide solution representing siRNA is recommended to avoid neutralization of cationic lipid by excess amount of anionic nucleotide and followed by agglomeration of liposome.

[*2] Less than 80 μl/1 mouse is recommended when no diluted solution is injected. Excess volume of injection might hurt animals. When dilution is required, use isotonic solution such as 9% sucrose solution or 5% glucose solution without containing ionized materials.

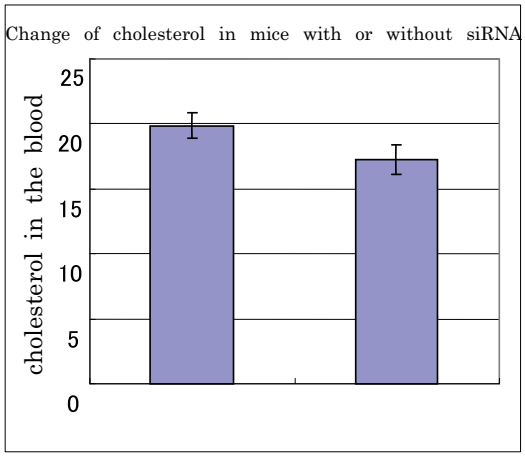
Example 1

Reduction of both mice serum cholesterol and LDL by injecting **LipoTrust™*EX* Oligo** / siRNA complex which can down-regulate Apo-B gene

Procedure

(1) 1 ml of prepared 83.0 pmol/μL of siRNA solution which can down-regulate Apo-B gene was poured into **LipoTrust™*EX* Oligo** vial. Liquid was mixed well and kept for 20 minutes according to the **Protocol.**
(2) Aliquot of each 80 μl were injected into 6 week old mice (n=5) from tail vein once in a day.
(3) The same volume of injection was continuously repeated for 3 days and 80 μl of blood were taken 48 hours after final injection.
(4) Blood was allowed for ultra-centrifugation with 10,000 rpm for 10 minutes.
(5) Serum was taken, and then both cholesterol and LDL were analyzed.
(6) For the comparison, 1 mL of **LipoTrust™*EX* Oligo** liposome without containing siRNA was prepared and injected into mice (n=5) with the same volume and same interval.

<Figure 1>



Result

As shown in the right figure, more than 10% reduction of both cholesterol and LDL using siRNA mixed liposome were observed in comparison with that of siRNA-free liposome.

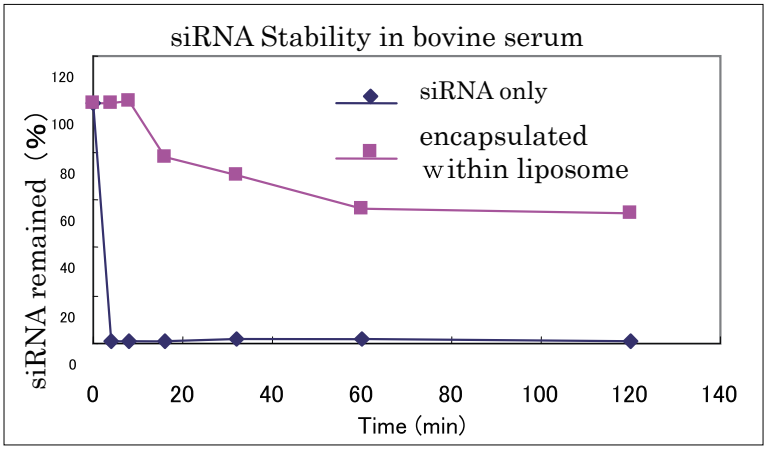
Advantage

Improvement of siRNA stability in the serum mixed with liposome

SiRNA easily decomposes in a few minutes by Nuclease in the presence of serum whereas siRNA mixed with appropriate liposome can survive for a few hours.

In fact, more than 50% of siRNA activity remained in 120 minutes with **LipoTrust™*EX* Oligo** while 99 % of naked siRNA decomposed less than 5 minutes in the presence of bovine serum as shown in Figure 2.

<Figure 2>



Both free siRNA and siRNA encapsulated in liposome were allowed to incubate in the bovine serum for more than 2 hours. Aliquots of serums were taken intermittently and respective RNAs were extracted .followed by siRNA contents analysis with HPLC.

As shown in the left Figure, free siRNA decomposed rapidly while as much as 50% of siRNA encapsulated in liposome were found to survive during 120 minutes incubation.

■ **Precautions for use**

-Liposomal suspension should be used as soon as possible for good results.

This product is distributed for laboratory research use only. Caution: Not for diagnostic, food, cosmetic or household use. Never inject human. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

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