


LipoTrust™ EX Oligo <in vitro>
 Hokkaido System Science Co., Ltd.

Contents: LipoTrust™ EX Oligo <in vitro>

Amount and Storage: Lyophilizate for 1ml use (containing 1µmol cationic lipid); one vial Storage at 2-8 C

Description: **LipoTrust™ EX Oligo <in vitro>** is a proprietary cationic liposome formulation that facilitates highly efficient delivery of **short oligonucleotides** such as **siRNA**, **antisense DNA** or **miRNA** to mammalian cells. Lyophilized powder is supplied so that customers can select 2 types of application which are **Lipoplex Type** and **Coating Type**. **Lipoplex Type** utilizes mixing of both separately prepared liposome suspension and **short oligonucleotides** solution while **Coating Type** utilizes direct mixing of **short oligonucleotides** solution into lyophilized powder.

TIP for Transfection

1. Although good transfection efficiency is observed with **LipoTrust™ EX Oligo <in vitro>** under serum containing medium (Ex:FEB 10%), use serum free medium at the time of preparation of Transfection complex.
2. Use healthy cells
3. Use medium free of antibiotics
4. Use high quality of oligonucleotide

Transfection Procedure**A. Lipoplex Type** : Method of mixing prepared liposome suspension using nuclease free water with nucleotide solution**A-1. Preparation of liposome suspension**

1. Remove the aluminum cap of vial by tool with extreme care and take off the rubber cap.
2. Add 1 mL of **nuclease free** water into the **LipoTrust™ EX Oligo** vial and gently shake the vial until no powder is remained. Be kept for 10 minutes before use. Please store this suspension at 2-8 .

A-2. Transfection

Use this procedure to transfect siRNA, antisense DNA or miRNA into mammalian cells using **LipoTrust™ EX Oligo** in a 24-well format. For other formats, see the table in **Recommended Reagent Amounts and Volumes** for appropriate reagent amounts to add.

Tip: To reduce well-to-well variability when transfecting multiple replicates (triplicates), proportionally scale up the reagent volumes to form complexes, then aliquot an equal volume of complexes into each well.

STEP 1. Cell plating

One day before transfection, plate cells and culture them for 1 day in ordinary growth medium without antibiotics per well. Cells should be 60-80% confluent at the time of transfection. (ex. HeLa Cell: 2-4x10⁴ cells/well)

STEP 2. Preparation of Transfection Complex and Application to Cells

1. Add 20 pmol of oligonucleotides in 50 µl of serum free medium previously prepared in a suitable microtube and mix well.
2. Add 2 µl of prepared **LipoTrust™ EX Oligo** suspension (**A-1**) and mix gently. (vortex mix with 5-10 seconds)
3. Incubate for 15 -20 minutes at room temperature to form **Transfection complex**.
4. Transfer this **complex** to well in which cells were plated (STEP 1) and mix well with gently rocking the plate back and forth.

STEP 3. Incubation

Incubate the cells at 37 for 24 to 72 hours.

Incubation time may be varied according to cells or oligonucleotides so that previous test to examine appropriate conditions is recommended.

Recommended Reagent Amounts and Volumes per 1 well

Step	1		2-1		2-2
	Medium volume/well	Serum-free medium dilution for oligonucleotide	Oligonucleotide* ¹	LipoTrust™ EX Oligo* ¹	
96 well	100 µl	10 µl	4pmol / 60ng ^{*2}	0.5 µl	
48 well	200 µl	20 µl	8pmol / 120ng	1 µl	
24 well	500 µl	50 µl	20pmol / 300ng	2 µl	
12 well	1 ml	100 µl	40pmol / 600ng	4 µl	
6 well	2.5 ml	250 µl	100pmol / 1.6µg	10 µl	
10 cm	15 ml	1.5 ml	600pmol / 9.2µg	60 µl	

*¹ Optimum amounts of **Oligonucleotides** and **LipoTrust™ EX Oligo** may be varied according to kind of cells or conditions.

Variation from 1/4 to 4 times of **LipoTrust™ EX Oligo** is recommended to obtain optimum transfection efficiency.

*² In case that Oligonucleotide is 21 mer and double helix RNA, this amount is suitable.

In case that plating and transfection is simultaneously performed, excess number of cells on plating is recommended.

B. Coating Type : Method of direct mixing prepared nucleotide solution with lyophilized lipid powder

B-1. Transfection

Use this procedure to transfect siRNA, antisense DNA or miRNA into mammalian cells using **LipoTrust™ EX Oligo** in a 24-well format. For other formats, see the table in **Recommended Reagent Amounts and Volumes** for appropriate reagent amounts to add.

Tip: To reduce well-to-well variability when transfecting multiple replicates (triplicates), proportionally scale up the reagent volumes to form complexes, then aliquot an equal volume of complexes into each well.

STEP 1. Cell plating

One day before transfection, plate cells and culture them for 1 day in ordinary growth medium without antibiotics per well. Cells should be 60-80% confluent at the time of transfection. (ex. HeLa Cell: $2-4 \times 10^4$ cells/well)

Serum contained medium is possibly used.

STEP 2. Preparation of Transfection Complex and Application to Cells

1. Remove the aluminum cap of **LipoTrust™ EX Oligo** vial by tool with extreme care and take off the rubber cap.
2. Add 1 ml of 20 μ M of oligonucleotides solution which was previously prepared into the vial and mix well. This suspension should be used within a day.
3. Incubate for 15 -20 minutes at room temperature to form **Transfection complex**.
4. Take 2 μ l of this **complex** and transfer into microtube containing 250 μ l serum free medium and mix well with gentle.
5. Add 250 μ l of 20% serum contained medium into microtube and mix well.
6. Remove medium from cell plating (**STEP 1**) and transfer 500 μ l of prepared mixture at **STEP 2-5**.

STEP 3. Incubation

Incubate the cells at 37 °C for 24 to 72 hours.

Incubation time may be varied according to cells or oligonucleotide so that previous test to examine appropriate conditions is recommended.

Recommend Reagent Amounts and Volumes per 1 well (20 μ M oligonucleotide solution is used)

Step	1	2-1		2-2
Plate/dish	Medium volume/well	Serum-free medium dilution for oligonucleotide	LipoTrust™ EX Oligo ^{*3}	20% serum contained medium
96well	100 μ l	50 μ l	0.5 μ l	50 μ l
48well	200 μ l	100 μ l	1 μ l	100 μ l
24well	500 μ l	250 μ l	2 μ l	250 μ l
12well	1ml	500 μ l	4 μ l	500 μ l
6well	2.5ml	1.3 ml	10 μ l	1.3 ml
10 cm	15 ml	7.5 ml	60 μ l	7.5 ml

^{*3} Optimum amounts of **Oligonucleotides** and **LipoTrust™ EX Oligo** may be varied according to kind of cells or conditions. Variation from 1/4 to 4 times of **LipoTrust™ EX Oligo** is recommended to obtain optimum transfection efficiency.

(Ex: While 1/4 times of **LipoTrust™ EX Oligo**, 80 μ M of oligonucleotide solution is appropriate. In case of 2 times, 10 μ M is suitable.)

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For any inquiry about the product, please contact to

Hokkaido System Science Co., Ltd.
2-1, Shinkawa Nishi 2-1, Kitaku
Sapporo, Hokkaido, Japan 001-0932
TEL: 81-11-768-5901 FAX: 81-11-768-5951
E-mail: bio@hssnet.co.jp
URL: <http://www.hssnet.co.jp>

