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**. **Lypoplex 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: 2-4x10^4 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 **Trasfection 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
Plate/dish	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: 2-4x10^4 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 μM 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 **Trasfection complex**.
4. Take 2μl of this **complex** and transfer into microtube containing 250 μl serum free medium and mix well with gentle.
5. Add 250 μl of 20% serum contained medium into microtube and mix well.
6. Remove medium from cell plating (**STEP 1**) and transfer 500 μl of prepared mixture at **STEP 2-5**.

STEP 3. Incubation

Incubate the cells at 37℃ 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 (20μM 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 μl	50 μl	0.5 μl	50 μl
48well	200 μl	100 μl	1 μl	100 μl
24well	500 μl	250 μl	2 μl	250 μl
12well	1ml	500 μl	4 μl	500 μl
6well	2.5ml	1.3 ml	10 μl	1.3 ml
10 cm	15 ml	7.5 ml	60 μl	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**, 80μM of oligonucleotide solution is appropriate. In case of 2 times, 10μM is suitable.)

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

For any inquiry about the product, please contact to

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