F09127 07/03/12 Rev.1

Gene / nucleic acid transfection reagent

LipoTrust™ Set

■Contents	■Amount and Storage
LipoTrust™ SR	Lyophilizate for 1ml use (containing 1μmol cationic lipid) ; one for each type
LipoTrust™ PE	Storage tube for the solution ; one for each type
LipoTrust™ CH	Storage at 2-8 degrees

■Description

As most transfection reagents have little gene expression activity in serum-containing medium, use of serum-free medium is recommended. **LipoTrust™** has enough gene expression activity in serum-containing medium as well, so that it can be used in both type of experiments with or without serum either *in vitro* or *in vivo*.

Though general transfection reagents are known to be cytotoxic, **LipoTrust™** causes much less cytotoxicity. Moreover, the product form, lyophilizate, makes it possible to choose from two types depend on your purpose. One is "Coating Type", which is to be used by dissolving the empty **LipoTrust™** with nucleic acid solution to reconstruct liposomal membrane without compromising effective nucleic acid retention. The other "Lipoplex Type" is the general type which is used by mixing the transfection reagent solution with nucleic acid solution.

LipoTrust™ SR : shows high gene expression activity to many kinds of cells in the presence of serum

LipoTrust™ PE : shows constant gene expression activity in the presence or absence of serum

LipoTrust™ CH : shows remarkably high gene expression activity in the serum-free medium, and is available for transfection into non-adherent type cells.

■Quality Control

LipoTrust™ is tested to be transfected to HeLa cell in both serum-free medium and 10% FBS-containing medium after the mix with β-gal gene possessing plasmid, then measured its β-gal-positive cell numbers.

■Cautions

- The lyophilized **LipoTrust™** (powdery state) and the dispersed **LipoTrust™** in water which has been made by adding distilled water to the lyophilized one should be stored at 2-8degrees in dark and cool place.
- Both "Coating Type" and "Lipoplex Type" are to be diluted when they are added to the cells at *in vitro* experiment. Use of the diluent like PBS (phosphate buffered saline) and cell culture medium (solution containing electrolyte such as NaCl) may yield the better result.
- **LipoTrust™** (especially SR and PE type) can show high gene expression effect even in the serum-containing medium (e.g. 10%FBS), which is very similar to the effect in the serum-free medium. High expression in the serum-free medium is confirmed for CH type. Transfection in the serum-free medium is recommended if the higher gene expression effect is necessary, as gene expression activity is generally higher in the serum-free medium than in the serum-containing medium. Optimum type of liposomes differs depending on cell types or experimental conditions. Please select and determine the liposome type necessary for users' own laboratory at the beginning.
- This product is sold only for research use. Do not use for the other purpose (medical, clinical diagnosis, food, cosmetic, household use etc.). DO NOT administer this product to human body.

■How to use

LipoTrust™ is the liposome formulation that packs the empty liposomes without gene in vial and is lyophilized. This enables two ways of use as follows.

【Coating Type】

Add nucleic acid solution to the lyophilized empty liposomes and mix gently. Then "Coating Type" liposomes containing nucleic acid will be produced. "Coating Type" means that the surface of nucleic acid is coated with liposomal membrane.

【Lipoplex Type】

Add distilled water to the lyophilized empty liposomes and mix gently. Then mix this liposomal dispersion with nucleic acid solution, and nucleic acid / liposomes complexes of "Lipoplex Type" will be produced. "Lipoplex Type" will show higher gene expression activity in *in vitro* experiment than "Coating Type".

■Transfection Protocol Example for "Coating Type"

Add 1 ml of 100 μg/ml nucleic acid solution[*1] to one vial of **LipoTrust™** (equivalent to 1μg of nucleic acid to 10 nmol of cationic lipid), then leave for 5 minutes. Then dilute it to be 1 μg/ml (for *in vitro* experiment) or 20 μg/ml (for *in vivo* experiment) of final nucleic acid concentration with serum-free medium (for *in vitro* or *in vivo* experiment) or 10% FBS-containing medium (*in vitro* experiment). [*2]

*1 : As **LipoTrust™** is designed to be isotonic at the dissolution by 1 ml of distilled water, please use distilled water as a solvent for nucleic acid solution as far as possible.

*2 : Saline and PBS are available instead of the above serum-free medium.

For any inquiry about the product, please contact to

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■Transfection Protocol Example for "Lipoplex Type"

Final nucleic acid concentration of 1 μg/ml (*in vitro* experiment) or 20 μg/ml (*in vivo* experiment)

	operation	*in vitro* experiment	*in vivo* experiment
Stock Sol.A	Dissolve **LipoTrust™** with 1ml of distilled water	1 ml (1 μmol/ml as a cationic lipid)	
Sol.A	*in vitro* experiment : Dilute 100μl of Stock Sol. A with 900μl of serum-free medium. *in vivo* experiment : Dilute 400μl of Stock Sol. A with 600μl of serum-free medium.	100nmol/ml as a cationic lipid	400nmol/ml as a cationic lipid
Sol.B	Prepare nucleic acid solution of the suggested concentration in serum-free medium	10μg/ml as nucleic acid	40μg/ml as nucleic acid
Sol.C	Mix the same amount of Sol.A and Sol. B (100μl each for *in vitro* experiment, 500μl each for *in vivo* experiment) to form the complex	1μg nucleic acid to 10nmol cationic lipid (total amount: 200μl)	20μg nucleic acid to 200nmol cationic lipid (total amount: 1ml)
−	Leave the complex to be stable	5 min	5 min
Final sample	*in vitro* : Add 800μl of serum-free or 12.5% FBS-containing medium to 200μl of Sol. C *in vivo* : use Sol. C directly	1μg/ml of final nucleic acid concentration (total amount 1 ml)	20μg/ml of final nucleic acid concentration (total amount 1 ml)

*3 : Saline and PBS are available instead of the above serum-free medium.

■Gene Expression Experiment Example

In vitro experiment example - Luciferase activity

Tumor cells ($1x10^5$- $8x10^5$) were seeded in a 6 well-plate to about 30-50% confluence, and washed once with serum-free medium or 10% FBS-containing medium after 24 hours incubation in 10% FBS-containing medium. Then 1ml of liposomal dispersion containing gene plasmid, pPGV-C (Coating Type and Lipoplex Type : 1μg/10nmol of cationic lipid / ml as a final DNA concentration), was added to each well, and 1-5 hours reaction was performed at 37 degrees. After the reaction, wells were washed once with serum-free medium or 10% FBS-containing medium, then cultured for 1-2 days in 10% FBS-containing medium. Then luciferase-assay was carried out. Luciferase-assay was carried out as follows. After having washed twice with PBS(-), the well was added 150μl of cell solution (LCβ) and left for 15 min, then scraped its surface with a cell scraper. This lysate was then centrifuged for two min at 12,000 r.p.m., 20μl of its supernatant was mixed with 100μl of luminescent reagent, and the light emission of the mixture was measured with lumiphotometer (TD-4000, Laboscience). Its protein amount was measured with BCA Protein Assay Reagent (Pierce) to estimate the Luciferase activity as the light emission amount per 1mg of protein.

In vitro experiment example - X-gal stain

Tumor cells ($1x10^5$- $8x10^5$) were seeded in a 6 well-plate to about 30-50% confluence, and washed once with serum-free medium or 10% FBS-containing medium after 24 hours incubation in 10% FBS-containing medium. Then 1ml of liposomal dispersion containing gene plasmid, pCAG-lacZ (Coating Type and Lipoplex Type : 1μg/10nmol of cationic lipid / ml as a final DNA concentration) was added to each well, and 1-5 hours reaction was performed at 37 degrees. After the reaction, wells were washed once with serum-free medium or 10% FBS-containing medium, then cultured for 1-2 days with 10% FBS-containing medium. Then X-gal stain was carried out.
X-gal stain was carried out as follows. After having washed once with PBS(-), the well was fixed for 3-4 min with PBS(-) containing 1% formaldehyde, 0.2% glutaraldehyde and 0.02% NP-40, then washed three times with PBS(-) for 10 min. Finally, the well was stained for 5-8 hours with the mixture of 5mM $K4[Fe(CN)_6]$, 5mM $K_3[Fe(CN)_6]$, 0.01% Sodium Deoxycholate, 0.02% NP-40, 2mM $MgCl_2$ and 0.1% X-gal. After the staining, the ratio of LacZ-positive cells was determined by microscopic count of more than 1,000 cells.

In vitro experiment example - X-gal stain (HeLa cell)

On the day before the transfection, $1x10^4$- $5x10^4$ HeLa cells were seeded in a 24 well-plate, and incubated for 24 hours in 10% FBS-containing medium.
Just before the transfection, the medium in the well was discarded and the well was washed once with serum-free medium or 10% FBS-containing medium. After that, according to the experiment method, 400μl of serum-free medium or 12.5% FBS-containing medium was added. Then 100μl of liposomal dispersion which contained the plasmid possessing β-gal gene (1μg/10nmol of cationic lipid / 100μl; sol.C) was added to each well, and 1 hour reaction was performed at 37 degrees. After 1 hour, 500μl of 20% or 10% FBS-containing medium was added to each well, and they were diluted to be 10% FBS-containing state. Then it was cultured for less than 24 hours at 37 degrees, and stained with X-gal.
X-gal stain was carried out as follows. After having washed once with PBS(-), the well was fixed for 3-4 min with PBS(-) containing 1% formaldehyde, 0.2% glutaraldehyde and 0.02% NP-40, then washed three times with PBS(-) for 10 min. Finally, the well was stained for 5-8 hours with the mixture of 5mM $K4[Fe(CN)_6]$, 5mM $K_3[Fe(CN)_6]$, 0.01% Sodium Deoxycholate, 0.02% NP-40, 2mM $MgCl_2$ and 0.1% X-gal. After the staining, the ratio of LacZ-positive cells was determined by microscopic count of more than 1,000 cells.

In vivo experiment example - X-gal stain

Tumor cells ($5x10^6$- $6x10^7$) were seeded in the abdominal cavity of nude mouse. After the period for 1day to 3 weeks (depending on the tumor cell types), 1ml of liposomal dispersion containing gene plasmid, pCAG-lacZ (Coating Type and Lipoplex Type : 20μg/200nmol of cationic lipid / ml as a final DNA concentration) was administered to the abdominal cavity of the mouse. After 1-2 days, each tumor cell was picked and $3x10^5$- $5x10^5$ cells were seeded in 6-well plate. When the cells were adhered after 24 hours incubation with 10% FBS-containing medium, X-gal stain was carried out. The way of X-gal stain was same as that of *in vitro* experiment.