NIBORLAB

LIPOTRANSFECTIN

Transfection Reagent for Mammalian Cells and more

Description	Cat. No.	Size
LIPOTRANSFECTIN	LTF-1	1.0 ml vial

Shipping: At room temperature (freeze immediately on receipt!)

Storage: ≤ –15°C

Stability: Best before (unopened): see label

Use: Only for research purposes *in vitro*, not intended for human or animal diagnostic, therapeutic or other clinical uses.

Description

LIPOTRANSFECTIN is the latest generation transfection reagent setting a new benchmark in mammalian cell transfection. Its novel composition combines **RMA**-Technology ("**R**epulsive **M**embrane **A**cidolysis") with a new **T**oxicity-**OP**timization Module (**TOP**-Technology), which regulates the lipoplex release of genetic material. On the one hand toxicity effects are minimized; on the other hand a maximum enhancement of concentration of naked gene in the cytosol and uptake in the nucleus is assured. Previous data support the fact that at least equivalent results can be achieved with LIPOTRANSFECTIN in comparison with LIPOTRANSFECTIN CLASSIC, but multiple enhancement of efficiency can be obtained eminently with moderately hard- or hard-to-transfect cell lines.

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1. General Information

1.1 Specifications

Application	Transfection of nucleic acids into mammalian cells
Formulation	Cationic lipids with colipids in water
Assays	up to 1000 (24-well) or up to 300 (6-well) with 1 ml reagent
Sterility	tested
Cell Culture	tested
Shelf Life	1 year
Storage	≤ –15°C

1.2 Quality Control

Standard transfection assay. Absence of bacterial and fungal contamination is verified using thioglycolate medium.

1.3 Explanatory Remarks

LIPOTRANSFECTIN is provided as a ready-to-use solution. It shows no serum inhibition, which makes it a reagent of choice for transfecting sensitive cell lines.

Storage

LIPOTRANSFECTIN is delivered non-chilled and should be stored in a freezer at approx. ≤ –15°C immediately after receipt. Freezing minimizes the natural ageing process of the liposomes.

Storage for several days at room temperature is not a problem provided that the reagent is subsequently stored again at ≤ –15°C; the number of times the product is thawed for use and re-frozen is irrelevant.

State of cells

Cells to be transfected should be well proliferating and healthy. Cells which have been in a quiescent state at confluency for a while (before seeding) may not be transfected as efficiently as cells which are growing rapidly (30 – 60% true confluency). Therefore it is recommended to use regularly passaged cells for transfection experiments. Microbial contamination, for example with mycoplasma or fungi, can drastically alter transfection results.

Antibiotics must be avoided where indicated. In some cases cell death can be caused by use of antibiotics in the transfection medium.

Cell confluency

The true confluency of the cells (adherent) to be transfected cannot be estimated visually by using a microscope, but can be optimally determined by means of a growing curve and comparison with counted cells

Note: The DNA transfection during the exponential growing phase of the cells is essential for optimum results, because of the critical role of cell division in transport of the DNA into the nucleus. The optimal confluency has to be adapted to the cell line used!

Good results are regularly obtained by using a covered growing area of 90 – 100% (visual confluency, see section 5 point 2). Usually transfection of siRNA is independent from cell division and requires a lower cell density at the point of transfection compared to DNA.

Optimization

Although LIPOTRANSFECTIN shows a broad peak performance, if optimal results are desired we recommend optimization of the transfection protocol for each combination of plasmid and cell line used. Every cell line has a characteristically optimal DNA/RNA/siRNA-lipid ratio. The format of dishes for lipoplex formation and cell culture can influence the ratio and absolute amounts of reagents.*

Additionally, a protocol used for other transfection reagents should never be transferred to LIPOTRANSFECTIN (or any other different transfection reagent). Every transfection reagent possesses its own molecular structure with specific physical properties, which have an important influence on DNA/RNA/siRNA-lipid ratios. Appropriate optimization instructions are given in section 3. As a rule, only little optimization is required if the recommended starting points are used.

DNA/RNA/siRNA should be of highest purity if optimal results for transfection are desired. For example, endotoxins decrease transfection efficiency. Before its use in complex formation with LIPOTRANSFECTIN, DNA/RNA/siRNA should not be stored diluted in medium for much longer than 5 min. Adsorption of DNA/RNA/siRNA in container materials can result in decrease of transfection efficiency. Polypropylene shows a minimum tendency towards adsorption of transfection reagent and genetic material in comparison to e.g. glass and polyethylene.

Note: RNA means single stranded RNA (ssRNA), not siRNA!

More information is available in section 3.

Stable transfection

If you desire stable transfection, follow the usual working instructions for seeding cells with lower density. On the day of transfection, cells should preferably be less than 50% confluent. After the transfection procedure, replace transfection medium with a suitable selected medium containing antibiotics.

* Probably caused by differing adsorption properties of the tube material based on differing surface dimensions.

2. Working Instructions

2.1 Transfection of adherent cells – Standard protocol for a 12-well plate

ⓘ **We recommend beginning with the starting points mentioned and optimizing by following the optimization protocol on page 10.**

1. In a 12-well tissue culture plate, seed 1.0 – 4.0 • 10^5 (starting point: 2.0 • 10^5) cells per dish in 1 ml of suitable fresh complete medium.*
2. Incubate the cells at 37°C in a CO_2 incubator until growing area is 90 – 100% covered (for details see point 3.2). The time required will vary among cell types, but will usually take 18 – 24 hours.
3. The stock solutions of the genetic material and the transfection reagent should be at room temperature. Agitate the stock solutions gently before use.
4. Prepare the following solutions using a cell culture grade 96-well plate or other tubes made of polypropylene, glass or polystyrene (preferentially polypropylene). **Medium must be pipetted first.** Pure solutions must not come into contact with plastic surfaces:

   Solution A: 0.5 – 1.5 µg of DNA(RNA) in 50 µl serum- and antibiotic-free medium or PBS (point 3.2).

   Solution B: 1.0 – 7.0 µl of LIPOTRANSFECTIN in 50 µl serum- and antibiotic-free medium or PBS (point 3.2).

☝ **Note: The DNA(RNA) lipid ratio has to be kept between 1:1 and 1:7 [µg DNA(RNA) : µl LIPOTRANSFECTIN]!**

Ratios will require optimizing based on various factors (see sections 3 and 4)

☝ **Note the order of addition: Add the DNA(RNA) solution into the transfection reagent solution and not in reverse!**

5. Mix the solutions gently by carefully pipetting one time.
6. Combine the two solutions, **mix gently by pipetting up and down once** and incubate at room temperature for 15 – 20 min.

☝ **Note: Shear stress may destroy the DNA(RNA) lipid complex as well as pure DNA!**

7. After incubation time add as soon as possible the DNA(RNA)-lipid complexes dropwise to the cells and swirl the flask with **extreme care**. Incubate at 37°C in a CO_2 incubator.†
8. Depending on cell type and promoter activity, assay cell extracts for gene activity 24 – 72 h following the start of transfection.

* Numbers of cells to seed depend on cell type and size. Optimization may be necessary, e.g. via determination of a growing curve. Maintain same seeding conditions between experiments.

† If toxicity is a problem because of very sensitive cells, remove the transfection mixture after 3 – 6 hours and replace it with medium.

2.2 Transfection of suspension cells – Standard protocol

1. In a 12-well tissue culture plate, seed 0.4 – 1.6 • 10^5 cells per dish in 1 ml suitable fresh complete medium.*
2. The stock solutions of the genetic material and the transfection reagent should be at room temperature. Agitate the stock solutions gently before use.
9. Prepare the following solutions using a cell culture grade 96-well plate or other tubes made of polypropylene, glass or polystyrene (preferentially polypropylene). **Medium must be pipetted first.** Pure solutions must not come into contact with plastic surfaces:

   Solution A: 0.5 – 1.5 µg of DNA(RNA) in
   50 µl serum- and antibiotic-free medium or PBS (point 3.2).

   Solution B: 1.0 – 7.0 µl of LIPOTRANSFECTIN in
   50 µl serum- and antibiotic-free medium or PBS (point 3.2).

Note: The DNA(RNA) lipid ratio has to be kept between 1:1 and 1:7 [µg DNA(RNA) : µl LIPOTRANSFECTIN]!

3. Ratios will require optimizing based on various factors (see sections 3 and 4)

Note the order of addition: Add the DNA(RNA) solution into the transfection reagent solution and not in reverse!

4. Mix the solutions gently by carefully pipetting one time.
5. Combine the two solutions, **mix gently by pipetting up and down once** and incubate at room temperature for 15 – 20 min.

Note: Shear stress may destroy the DNA(RNA) lipid complex as well as pure DNA!

6. After incubation time add as soon as possible the DNA(RNA)-lipid complexes dropwise to the cells and swirl the flask with **extreme care**. Incubate at 37°C in a CO_2 incubator.*
7. Depending on cell type and promoter activity, collect cells by centrifugation and assay cell extracts for gene activity 24 – 72 hours following the start of transfection.

* If toxicity is a problem because of very sensitive cells, remove the transfection mixture after 3 – 6 hours and replace it with medium.

2.3 Transfection of siRNA – Protocol for initial optimization in a 24-well plate

1. In a 24 well tissue culture plate, seed 0.1 – 1.0 • 10^5 cells per dish in 0.5 ml suitable fresh complete medium. For most cell types this range of cell amount will achieve the desired density of 30 – 50% visual confluency.*
2. The stock solutions of the genetic material and the transfection reagent should be at room temperature. Agitate the stock solutions gently before use.
3. Prepare the following solutions using a cell culture grade 96-well plate or other tubes made of polypropylene, glass or polystyrene (preferentially polypropylene) for each transfection. **Medium must be pipetted first.** Pure solutions must not come into contact with plastic surfaces:

Tube	**R1**	**R2**	**R3**	**R4**
siRNA	0.1 µg (ca. 7,5 pmol)	0.2 µg (ca. 15 pmol)	0.5 µg (ca. 40 pmol)	2 µg (ca. 150 pmol)
Add serum-free medium or PBS* volume	30 µl	30 µl	30 µl	30 µl
Tube	**M1**	**M2**	**M3**	**M4**
LIPOTRANSFECTIN	0.5 µl	1 µl	2.5 µl	10 µl
Add serum-free medium or PBS* volume	40 µl	40 µl	40 µl	40 µl

Note the order of addition: Add the DNA(RNA) solution into the transfection reagent solution and not in reverse!

4. Mix the solutions gently by carefully pipetting one time.
5. Combine the solutions R1 + M1, R2 + M2, R3 + M3, R4 + M4, **mix gently by pipetting up and down once** and incubate at room temperature for 15 – 20 min.
6. After incubation time add as soon as possible the siRNA-lipid complexes drop wise to the cells and **swirl the wells with extreme care** to ensure distribution overall. Incubate at 37°C in a CO2 incubator.

Note: Shear stress may destroy the DNA(RNA) lipid complex as well as pure DNA!

7. Depending on cell type, siRNA, stability of mRNA and the protein being targeted, assay for gene knockdown 24 – 72 hours following the start of transfection. Once the siRNA-lipid complex has been added to the cells, there is no need to remove it and replace with fresh medium.†

Note: To obtain the highest efficiency and low non-specific effects, optimize transfection conditions by varying siRNA and lipid concentrations.

* Numbers of cells to seed depend on cell type and size. Optimization may be necessary, e.g. via determination of a growing curve. Maintain same seeding conditions between experiments.

† If toxicity is a problem because of very sensitive cells, remove the transfection mixture after 3 – 6 hours and replace it with medium. When using serum-free medium during siRNA transfection procedure, replace the complex-containing medium with serum-containing medium 3 – 6 hours after start of transfection.

2.4 Co-Transfection Experiments (siRNA-DNA)

LIPOTRANSFECTIN works well in co-transfection of short inhibitory RNA and plasmid DNA. Usually transfection of plasmids requires a higher cell density at the point of transfection compared to siRNA. Note following recommendations:

1. Plate cells are as those described for transfection of plasmid DNA.
2. Maintain the same total lipid - total nucleic acid ratio as that used for siRNA alone. If you need to increase the total amount of nucleic acid, increase the amount of LIPOTRANSFECTIN in proportion to the total amount [µg] of nucleic acid.
3. Always use a volume of LIPOTRANSFECTIN [µl] that is at least double the total final mass of nucleic acid [µg].

3. Optimization

3.1 Critical optimization parameters

Ratio of LIPOTRANSFECTIN to DNA/RNA/siRNA

The most important optimization parameter is the ratio of DNA/RNA/siRNA to LIPOTRANSFECTIN.

For successful transfection a slightly net positive charge of the DNA/RNA/siRNA–LIPOTRANSFECTIN–complex is required. The optimal DNA/RNA/siRNA–LIPOTRANSFECTIN–ratio depends on the cell line.

Quantity of transfection complex

In order to obtain the highest transfection results, optimization of the absolute amount of DNA/RNA/siRNA-lipid-complex may be required.

Optimal ratio of LIPOTRANSFECTIN – DNA/RNA/siRNA and concentration of DNA/RNA/siRNA-lipid-complex may vary with the number of cells. An excessive amount of the complex can lead to over expression and/or lysis of cells (lipids are also lysis reagents!) It is necessary to keep the number of seeded cells and incubation period constant until the transfection procedure for a reproducible optimization of these parameters.

Numbers of cells to seed

For details see section 5 point 2.

Effect of serum

As yet, nearly all cell lines transfected with LIPOTRANSFECTIN have shown superior results if transfection is performed in the presence of serum. Nevertheless, special cell lines may show different behaviour. Accordingly transfection can be performed without serum, under serum-reduced or full-serum (e.g. 10%) condition.

Note: Presence of serum during complex formation between LIPOTRANSFECTIN and DNA/RNA/siRNA is strictly forbidden!

Serum may inhibit complex formation. Once the complex is formed, contact with serum is permitted.

Optimal ratio of LIPOTRANSFECTIN to DNA/RNA/siRNA and concentration of DNA/RNA/siRNA-lipid-complex may vary with different serum concentrations.

Optimization of these critical parameters by following the optimization instructions (see chapter 3.3) below should give satisfactory results.

3.2 Further optimization parameters

These parameters can be further optimized by a step-by-step procedure:

Incubation time with transfection complex

Cells can be exposed to the transfection complex within a variety of time ranges (i.e. 3 – 72 hours). Depending on the sensitivity of the transfected cell line, short or long exposure is possible.

Time range between transfection and evaluation

Assay for gene activity should be performed 24 – 72 hours after the start of transfection. The optimal time is dependant on cell type, promoter activity and expression product (e.g. toxicity).

Using PBS in DNA-lipid complex formation instead of serum-free medium

Numerous experiments showed that the use of 1x PBS in DNA/RNA/siRNA-lipid complex formation instead of serum- and antibiotic-free medium delivers transfection rates with improved

reproducibility and in some cases higher transfection rates, particularly with lower volumes of lipids. (see chapter 6.1)

PBS composition:

10x PBS (10x = 10-fold concentration):

40 g	NaCl
1 g	KCl
1 g	KH_2PO_4
5.75 g	$Na_2HPO_4 \bullet 2\ H_2O$

The salts are weighed, mixed, topped up to 500 ml with non-sterile water and dissolved, then autoclaved for 35 minutes at 121°C.

1x PBS:

100 ml 10x PBS is diluted 1:10 in a volumetric flask and autoclaved for 35 minutes at 121°C.

3.3 Optimization instructions

for siRNA applications see section 2.3

For optimizing purposes, use reporter gene plasmids such as pCMVßGal, pND2Luc, pEGFP etc.

1. Follow the working instructions, varying the amount of LIPOTRANSFECTIN within the interval proposed in the table in section 4 (e.g. 2 µl, 4 µl, 6 µl, 8 µl, 10 µl, 12 µl etc.). Keep the number of cells at the beginning of the transfection procedures and the DNA(RNA) amounts constant at the recommended starting-points. The serum concentration during incubation with the DNA(RNA)-lipid-complexes should be the same as the concentration with which the cells are cultured.
2. Follow the working instructions, varying the quantity of the DNA(RNA) (e.g. 1 µg, 1.5 µg, 2 µg, 2.5 µg, 3 µg etc.) and keeping the proposed interval of LIPOTRANSFECTIN proportional (see step 1). Also, keep the number of cells at the beginning of the transfection procedures constant at the recommended starting-point. The serum concentration during incubation with the DNA(RNA)-lipid-complexes should be the same as the concentration with which the cells are cultured. Determine the optimal DNA(RNA) and lipid amounts.
3. Repeat steps 1 and 2 with serum-reduced and serum-free conditions.
4. Repeat steps 1 und 2 with other starting-points for the number of cells at the beginning of the transfection procedures.

Example for a 12-well format

1. In a 12-well tissue culture plate, seed 1.0 – 4.0 • 10^5 (starting point: 2.0 • 10^5) cells per dish in 1 ml of suitable fresh complete medium.*
2. Incubate the cells at 37°C in a CO_2 incubator until growing area is 90 – 100% covered (for details see point 3.2). The time required will vary among cell types, but will usually take 18 – 24 hours.
3. The stock solutions of the genetic material and the transfection reagent should be at room temperature. Agitate the stock solutions gently before use.
4. Pipet 50 µl serum- and antibiotic-free medium or PBS (point 3.2) to each below stated well using a cell culture grade 96-well plate. Following add:

0,5 µg	DNA(RNA) in A1 – A4
1,0 µg	DNA(RNA) in B1 – B4
1,5 µg	DNA(RNA) in C1 – C4

   Mix the solutions gently by carefully pipetting one time.

5. Pipet 50 µl serum- and antibiotic-free medium or PBS (point 3.2) to each below stated well using a cell culture grade 96-well plate. Following add:

* Numbers of cells to seed depend on cell type and size. Optimization may be necessary, e.g. via determination of a growing curve. Maintain same seeding conditions between experiments.

1 µl, 2 µl, 4 µl, 6 µl	LIPOTRANSFECTIN in D1 – D4
2 µl, 4 µl, 8 µl, 12 µl	LIPOTRANSFECTIN in E1 – E4
4 µl, 8 µl, 12 µl, 16 µl	LIPOTRANSFECTIN in F1 – F4

Mix the solutions gently by carefully pipetting one time

Note the order of addition: Add the DNA(RNA) solution into the transfection reagent solution and not in reverse!

6. Combine the corresponding wells (A1 + D1, A2 + D2 etc., B1 + E1, B2 + E2 etc., C1 + F1, C2 + F2 etc.), **mix gently by pipetting up and down once** (shear stress can destroy the developing complex!) and incubate at room temperature for 15 – 20 min.

Note: Shear stress may destroy the DNA(RNA) lipid complex as well as pure DNA!

7. After incubation time add as soon as possible the DNA(RNA)-lipid complexes dropwise to the cells and swirl the flask with **extreme care**. Incubate at 37°C in a CO_2 incubator.*
8. Depending on cell type and promoter activity, assay cell extracts for gene activity 24 – 72 h following the start of transfection.

If results are satisfactory, scale up or down to other desired vessel sizes. In this case see table on following section 4.

* If toxicity is a problem because of very sensitive cells, remove the transfection mixture after 3 – 6 hours and replace it with medium.

4. Up- and Downscale

Reagent quantities for different sizes of culture vessels (proposed starting points for optimization in brackets).

1. In the majority of cases, the optimum ratio range of DNA/RNA/siRNA [µg] to LIPOTRANSFECTIN [µl] is between 1:2 and 1:7.
2. For siRNA applications use special siRNA optimization protocol. Adapt to your used size of wells by appropriate proportional factor of growth area relative to 24-well plate.
3. Adsorption processes of the vessel material with the agents used necessitate optimization of the amount of lipoplex and the DNA/RNA/siRNA-lipid ratio for each change to a significantly different format.

Culture plate diameter [mm]	**7** (96-well plate)	**16** (24-well plate)	**22** (12-well plate)	**35** (6-well plate)	**60**	**100**
Growth areas [cm^2]	0.31	1.9	3.7	9	22	60
Proportional factors	0.03	0.2	0.4	1.0	2.5	6.7
Adherent cells to seed* (day before transfection) [$\times 10^5$]	0.10 – 0.60 (0.30)	0.4 – 2.0 (1.0)	1.0 – 4.0 (2.0)	2.5 – 10.0 (5.0)	6.0 – 24.0 (12.0)	15.0 – 60.0 (25.0)
Suspension cells to seed* (day of transfection) [$\times 10^5$]	0.04 – 0.24 (0.12)	0.16 – 0.8 (0.40)	0.4 – 1.6 (0.8)	1.0 – 4.0 (2.0)	2.4 – 9.6 (4.8)	6.0 – 24.0 (10.0)
Cell suspension volume [ml]	0.15	0.5	1.0	2.0	4.5	12.0
DNA(RNA) amount [µg]	0.04 – 0.3 (0.1)	0.08 – 1.0 (0.5)	0.2 – 2.0 (1.0)	0.4 – 5.0 (2.0)	0.8 – 12.0 (6.0)	1.6 – 34.0 (14.0)
LIPOTRANSFECTIN - amount [µl]	0.2 – 4.0 (0.6)	0.4 – 7.0 (2.0)	0.8 – 15.0 (3.0)	1.6 – 35.0 (6.0)	3.2 – 90.0 (18.0)	6.4 - 250 (42.0)
Dilution volume of DNA(RNA) [µl]	15 - 30	30	50	100	300	700
Dilution volume of LIPOTRANSFECTIN [µl]	10 - 50	10 - 50	50	100	300	700
Total volume [ml]	0.175 – 0.23	0.54 – 0.58	1.1	2.2	5.1	13.4

Optimum DNA to LIPOTRANSFECTIN ratios [µg:µl] obtained for 24-well format

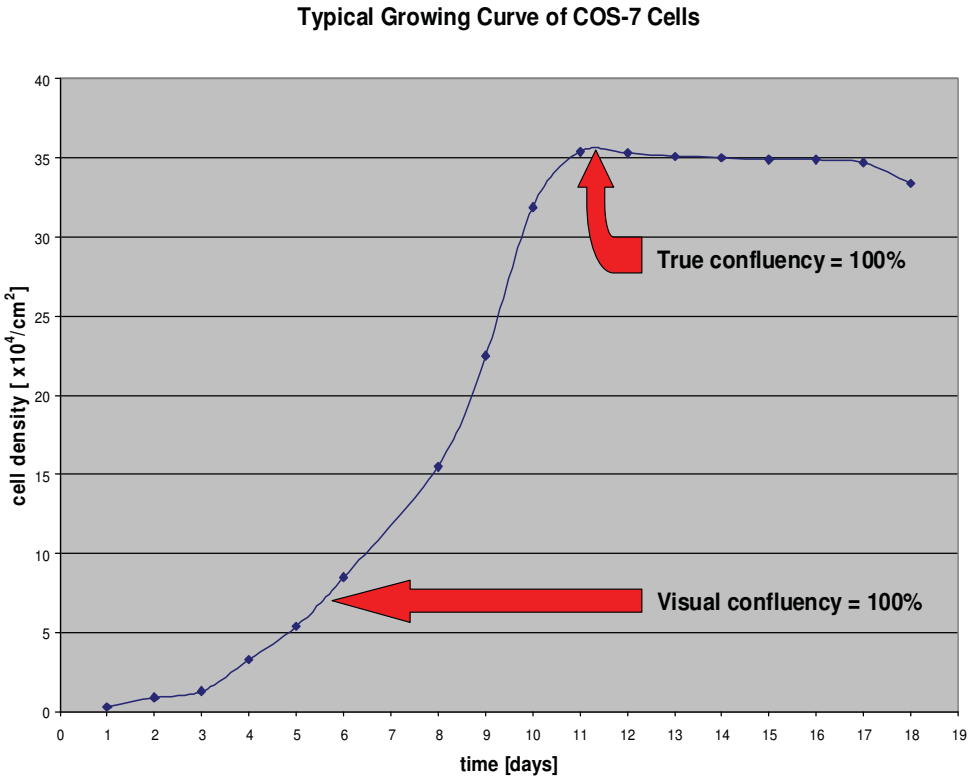
Cell line	**DNA amount used per well**		
	0.1 µg	**0.2 µg**	**0.3 µg**
BHK	1:3	1:3	1:2
CV-1	1:3	1:3	1:3
COS-7	1:2	1:2	1:2
MDCK	1:3	1:3	1:3

* Numbers of cells to seed depend on cell type and size. Optimization may be necessary, e.g. via determination of a growing curve. Maintain same seeding conditions between experiments. Usually transfection of plasmids requires a higher cell density at the point of transfection compared to siRNA (see optimization protocol for siRNA transfection.

5. Troubleshooting

for further information see:
http://www.niborlab.com

1. Avoid any contact of pure LIPOTRANSFECTIN and of pure DNA/RNA/siRNA solution with tube materials (e.g. 96 well plates).
   Conclusion: Serum- and antibiotics-free medium must be pipetted first! Diluted solutions should be combined for complex formation within 5 min!
2. Confluency determined visually ("visual" confluency = percentage of growth surface covered with cells) is **not identical** with confluency determined by growing curve (= true confluency). Best results are obtained if transfection is performed at the highest possible proliferation state (= 30 – 60% true confluency). This often corresponds with visual confluency of 90 – 100%.



3. DNA/RNA/siRNA- and LIPOTRANSFECTIN solutions diluted in medium should be combined within 5 min.
4. Decreased cell growth or toxicity is often associated with very high transfection activity (over expression). This effect can be avoided by transfection of cultures with higher confluency or with a lower amount of LIPOTRANSFECTIN – DNA/RNA/siRNA complex.
5. Do not add antibiotics to media during transfection as this may cause cell death and decrease transfection efficiency.
6. In case of very sensitive cells, remove the transfection mixture after 3 – 6 h and replace it with complete medium.

6. Miscellaneous

6.1 Buffer

10x PBS (10x = 10-fold concentration)			
40 g	NaCl	1 g	KH_2PO_4
1 g	KCl	5.75 g	$Na_2HPO_4 \cdot 2\ H_2O$
The salts are weighed, mixed, topped up to 500 ml with non-sterile water and dissolved, then autoclaved for 35 minutes at 121°C			
1x PBS			
100 ml 10x PBS is diluted 1:10 in a volumetric flask and autoclaved for 35 minutes at 121°C.			

6.2 Important Information

LIPOTRANSFECTIN is developed and sold for research purposes and in vitro use only. It is not intended for human therapeutic or diagnostic purposes. Appropriate care should be exercised when handling many of the reagents described in this publication.

6.3 Warranty

Niborlab guarantees the performance of the LIPOTRANSFECTIN, when used in accordance with the information given in this publication, for a period of 12 months from the date of purchase. If you are not completely satisfied with the performance of the product please contact Niborlab or one of its authorized distributors.

Niborlab

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