

XpressFect

Catalog Number	Size
XF20-1	1.0 ml
XF20-2	2 x 1.0 ml
XF20-5	5 x 1.0 ml

Description:

XpressFect is a polycationic transfection reagent based on liposome technology. The specifically designed molecular structure of the cationic lipid ensures easy entry of DNA or RNA into mammalian cells by condensing DNA or RNA into compact structures (DNA or RNA – lipid complex). In the cell the release of DNA or RNA is guaranteed by the built-in endosome buffering.

XpressFect is provided as a ready-to-use solution. It shows no serum inhibition, which makes it ideal for transfection of sensitive cell lines.

Application: Transfection of nucleic acids into mammalian cells

Formulation: Cationic lipids with co-lipids in water

Assays: Up to 1500 (24-well) or up to 400 (6-well) with 1 ml reagent

Shipping: Shipped at room temperature.

Storage: +4°C

Stability: See label for expiration date.

Formulations of liposomes like XpressFect change their size distribution after long storage at +4°C, which can have slight adverse effects on the transfection efficiency. This effect can be reversed by a freeze-thaw cycle. It is recommended to perform a freeze-thaw cycle before first use, and subsequently monthly to yield optimal results.

Note: This product is for research use only. Not for use in human or animal diagnostics, therapeutics, or clinical applications.

General Guidelines

State of Cells

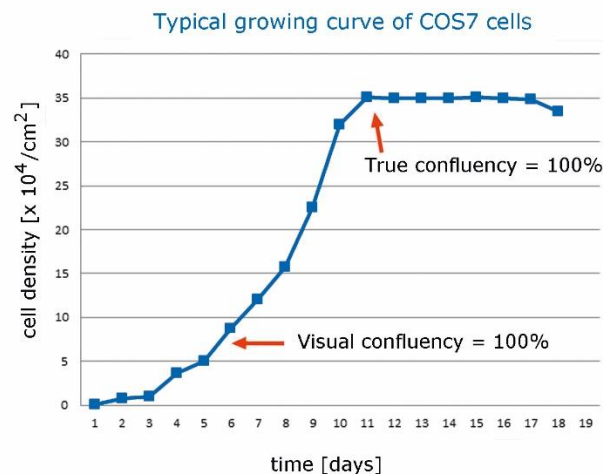
Cells to be transfected should be well proliferating and healthy. Cells which have been in a quiescent state at confluency for a while (before seeding) may not be transfected as efficiently as cells which are growing rapidly. Therefore, it is recommended to use regularly passaged cells for

transfection experiments. Microbial contamination with mycoplasma or fungi can drastically alter transfection results.

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.

Confluency determined visually ("visual" confluency = % of growth surface covered with cells) is **not identical** with confluency determined by a 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%.



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.

Quality of the Nucleic Acid

DNA or RNA should be of the highest purity if optimal results for transfection are desired. For example, endotoxins decrease transfection efficiency. Before its use in complex formation, DNA or RNA should not be stored diluted in medium for much longer than 5 minutes. Adsorption of DNA or RNA 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 glass and polyethylene.

Antibiotics

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

Optimization

XpressFect shows a broad peak performance, but if optimal results are desired it is recommended to optimize the transfection protocol for each combination of plasmid and cell line used. Every cell line has a characteristically optimal DNA or RNA-lipid ratio. The format of dishes for lipoplex formation and cell culture can influence the ration and absolute amounts of reagents (likely caused by differing adsorption properties of the tube material based on differing surface dimensions).

Additionally, a protocol used for other transfection reagents should never be transferred to XpressFect 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 or RNA-lipid ratios. Appropriate optimization instructions are given in this manual. As a rule, only little optimization is required if the recommended starting points are used.

Stable Transfection

If stable transfection is desired, 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.

Transfection Protocol

Optimization - determination of a growth curve may be necessary, please see Optimization Protocol.

Transfection of Adherent Cells (standard protocol for 12-well format)

1. In a 12-well tissue culture plate, seed $1.0 - 4.0 \times 10^5$ (starting point: 2.0×10^5) cells per dish in 1 ml of suitable fresh complete medium. The number of cells to seed depends on cell type and size. Optimization may be necessary, see Optimization Protocol. Maintain same seeding conditions between experiments.
2. Incubate the cells at 37°C in a CO₂ incubator until the growing area is 90 – 100% covered. The time required will vary among cell types but will usually take 18 – 24 hours.
3. The DNA or RNA stock solutions and transfection reagent should be at room temperature. Agitate the stock solutions gently before use.
4. Prepare the following solutions using a call culture grade 96-well plate or other tubes made of polypropylene, glass, or polystyrene (preferably 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 or RNA to 50 µl serum and antibiotic free medium or 1 x PBS.
Solution B: 1.0 – 7.0 µl of XpressFect to 50 µl serum and antibiotic free medium or 1 x PBS.
Note: The DNA or RNA-lipid ratio has to be kept between 1:2 and 1:7 (µg of DNA or RNA: µl of XpressFect).
5. Mix each solution gently by carefully pipetting one time.
6. Add **Solution A to Solution B** and mix carefully (shear stress may destroy the DNA or RNA-lipid complex). Incubate at room temperature for 15 – 20 minutes.
Note: The DNA or RNA solution (Solution A) MUST be added to the XpressFect solution (Solution B). Do not add Solution B to Solution A.

7. After incubation immediately add the DNA or RNA-lipid complex drop wise to the cells and swirl the flask with **extreme care**. If toxicity is a problem due to sensitive cells, remove the transfection mixture after 3 – 6 hours and replace it with medium. Incubate at 37°C in a CO₂ incubator.
8. Depending on the cell type and promoter activity, assay cells for gene activity 24 – 72 hours following the start of transfection.

Transfection of Suspension Cells (standard protocol for 12-well format)

1. In a 12-well tissue culture plate, seed 0.4 – 1.6 x 10⁵ cells per dish in 1 ml suitable fresh complete medium. The number of cells to seed depends on the cell type and size. Optimization may be necessary, see Optimization Protocol. Maintain same seeding conditions between experiments.
2. The DNA or RNA stock solutions and 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 tubes made of polypropylene, glass or polystyrene (preferably polypropylene) for each transfection. **Medium must be pipetted first.** Pure solutions must not come into contact with plastic surfaces.
Solution A: 0.5 – 1.5 µg of DNA or RNA to 50 µl serum and antibiotic free medium or 1 x PBS.
Solution B: 1.0 – 7.0 µl of XpressFect to 50 µl serum and antibiotic free medium or 1 x PBS.
Note: The DNA or RNA-lipid ratio has to be kept between 1:2 and 1:7 (µg of DNA or RNA: µl of XpressFect).
4. Mix each solution gently by carefully pipetting one time.
5. Add **Solution A to Solution B** and mix carefully (shear stress may destroy the DNA or RNA-lipid complex). Incubate at room temperature for 15 – 20 minutes.
Note: The DNA or RNA solution (Solution A) MUST be added to the XpressFect solution (Solution B). Do not add Solution B to Solution A.
6. After incubation immediately add the DNA or RNA-lipid complex drop wise to the cells and swirl the flask with **extreme care**. If toxicity is a problem due to sensitive cells, remove the transfection mixture after 3 – 6 hours and replace it with medium. Incubate at 37°C in a CO₂ incubator.
7. Depending on the cell type and promoter activity, assay cells for gene activity 24 – 72 hours following the start of transfection.

Optimization

Critical Optimization Parameters

Ratio of DNA or RNA to XpressFect

The most important optimization parameter is the ratio of DNA or RNA to XpressFect. For successful transfection a slightly net positive charge of the DNA or RNA- XpressFect complex is required. The optimal DNA or RNA- XpressFect 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- or RNA- lipid complex may be required.

Optimal ratio of DNA or RNA to XpressFect and concentration of DNA or RNA-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.

Effects of serum

To date, nearly all cell lines transfected with XpressFect have shown superior results if transfection is performed in the presence of serum. Special cell lines may show different behavior. Transfection can be performed without serum, under serum reduced, or full serum (e.g. 10%) conditions.

There must be no serum present during **complex formation** between XpressFect and DNA or RNA. Serum may inhibit complex formation. Once the complex is formed, contact with serum is permitted.

Optimal ratio of DNA or RNA to XpressFect and concentration of DNA- or RNA-lipid complex may vary with different serum concentrations. Optimization of these critical parameters by following the optimization protocol should give satisfactory results.

Additional 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 dependent on cell type, promoter activity and expression product (e.g. toxicity).

Using PBS instead of serum-free medium

Numerous experiments showed that the use of PBS in DNA or RNA-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.

PBS composition:

10X PBS

40 g NaCl
1 g KCl
1 g KH_2PO_4
5.75 g $\text{Na}_2\text{HPO}_4 \cdot 2 \text{H}_2\text{O}$

The salts are weighed, mixed, topped up to 500 ml with water and dissolved, then autoclaved.

1X PBS

10X PBS diluted 1:10 with water in a volumetric flask and autoclaved.

Addition of the transfection complex to freshly seeded cells

Addition of the transfection complex to adherent cells immediately (within 1 hour) after their seeding into adequate culture plates can result in a considerable increase of transfection efficiencies. It is not necessary to change the incubation time. **This procedure can shorten the time of a transfection experiment by 24 hours.**

Optimization Protocol

For optimizing purposes, use reporter gene plasmids such as pCMV β Gal, pND2Lux, pEGFP etc.

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

Example for 12-well format

1. In a 12-well microtiter plate, seed $1.0 - 4.0 \times 10^5$ cells per dish in 1 ml of suitable complete medium. (Numbers of cells to seed depend on the cell type and size. Optimization may be necessary. Maintain same seeding conditions between experiments).
2. Incubate the cells at 37°C in a CO₂ incubator. The growing area should be 90 – 200% covered. The time required will vary among cell types but will usually take 18 – 24 hours.
3. The solutions of the reporter gene DNA or RNA and XpressFect transfection reagent should be at ambient temperature and should be mixed gently prior to use.

4. Pipette 50 µl serum and antibiotic-free medium or 1X PBS to each well indicated below using a cell culture grade 96-well plate. Then add the following:

A1 – A4 0.5 µg DNA or RNA

B1 – B4 1.0 µg DNA or RNA

C1 – C4 1.5 µg DNA or RNA

Mix each solution gently by carefully pipetting one time.

5. Pipette 50 µl serum and antibiotic-free medium or 1X PBS to each below stated well using cell culture grade 96-well plate. Then add the following:

D1 – D4 1 µl, 2 µl, 4 µl, 6 µl XpressFect

E1 – E4 2 µl, 4 µl, 8 µl, 12 µl XpressFect

F1 – F4 4 µl, 8 µl, 12 µl, 16 µl XpressFect

Mix each solution gently by carefully pipetting one time.

6. Combine the corresponding wells (A1 + D1, A2 + D2, etc., B1 + E1, B2 + E2, etc., C1 + F1, C2 + F2, etc.), mix carefully (shear stress can destroy the developing complex) and incubate at room temperature for 15 – 20 minutes.

Note: The DNA or RNA solution from step 4 MUST be added to the XpressFect solution from step 5. DO NOT add the XpressFect solution to the DNA or RNA solution.

7. Immediately after incubation add the DNA or RNA-lipid complexes drop wise to the cells and swirl the flask with **extreme care**. Incubate at 37°C in a CO₂ incubator. If the toxicity is a problem because of highly sensitive cells, remove the transfection mixture after 3 – 6 and replace with medium.
8. Depending on cell type and promoter activity, assay cells for gene activity 24 – 72 hours following the start of transfection.

If the results are satisfactory, scale up or down to other desired vessel sizes. Please see the Up and Down Scale section on the following page.

Up and Downscale

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

Note:

1. In a majority of cases, the optimum ratio range of DNA or RNA (μg) to XpressFect (μl) is between 1:2 and 1:7.
2. Adsorption processes of the vessel material with the agents used necessitate optimization of the amount of lipoplex and the DNA or RNA-lipid ratio for each change to a significantly different format.

Proposed starting points for optimization in parenthesis.

Culture plate	96-well plate	24-well plate	12-well plate	6-well plate	60mm	100mm
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 (1 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 or RNA amount [μg]	0.05–0.3 (0.1)	0.15–1.0 (0.5)	0.3–2.0 (1.0)	0.4–5.0 (2.0)	1.0–12.0 (6.0)	2.0–34.0 (14.0)
XpressFect - amount [μl]	0.2–4.0 (0.6)	0.5–7.0 (2.0)	1.0–15.0 (3.0)	2.0–35.0 (6.0)	4.0–90.0 (18.0)	10–250 (42.0)
Dilution volume of DNA or RNA [μl]	15 - 30	30	50	100	300	700
Dilution volume of XpressFect [μl]	10 - 50	10 - 50	50	100	300	700

*Numbers of cells to seed depend on cell type and size. Optimization may be necessary. Maintain same seeding conditions between experiments.