Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

PRO-XpressFect

Catalog Number	Size
PROXF40-1	1.0 ml
PROXF40-2	2 x 1.0 ml
PROXF40-5	5 x 1.0 ml

Description:

PRO-XpressFect is a liposome-based transfection reagent consisting of a mixture of polycationic and neutral lipids. It is an advanced product based on XpressFect with at least equivalent results when compared side by side. Structural changes of the comprising cationic lipids results in higher efficiency and lower toxicity, making PRO-XpressFect an ideal choice for moderately hard or hard-to-transfect cell lines.

PRO-XpressFect is provided as a ready-to-use solution. It shows no serum inhibition, making it a reagent of choice for transfecting 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 PRO-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

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

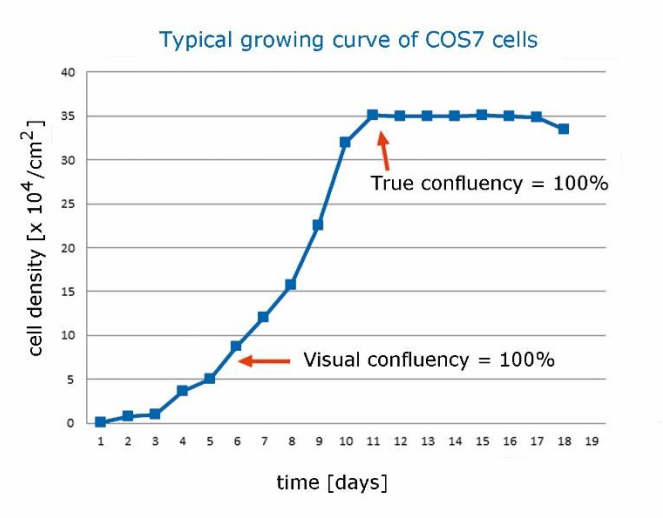
Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

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.

Usually, transfection of siRNA is independent from cell division and requires a lower cell density at the point of transfection compared to DNA.

Quality of the Nucleic Acid

Nucleic acid 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, nucleic acid should not be stored diluted in medium for much longer than 5 minutes. Adsorption of nucleic acid 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.

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

Optimization

PRO-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 nucleic acid-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 PRO-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 x 10^5 (starting point: 2.0 x 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 CO_2 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.
   Note: RNA means single-stranded RNA (ssRNA), not siRNA.
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 PRO-XpressFect to 50 µl serum and antibiotic free medium or 1 x PBS.

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

Note: The DNA or RNA-lipid ratio has to be kept between 1:2 and 1:7 (µg of DNA or RNA: µl of PRO-XpressFect).

5. Mix each solution gently by carefully pipetting one time.
6. Add **Solution A to Solution B** and mix carefully by pipetting up and down once (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 PRO-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**. Incubate at 37°C in a CO_2 incubator.
   Note: If toxicity is a problem due to sensitive cells, remove the transfection mixture after 3 – 6 hours and replace it with medium.
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 10^5 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 PRO-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 PRO-XpressFect).
4. Mix each solution gently by carefully pipetting one time.
5. Add **Solution A to Solution B** and mix carefully by pipetting up and down once (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 PRO-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**. Incubate at 37°C in a CO_2 incubator.
   Note: If toxicity is a problem due to sensitive cells, remove the transfection mixture after 3 – 6 hours and replace it with medium.
7. Depending on the cell type and promoter activity, collect cells by centrifugation and assay cells for gene activity 24 – 72 hours following the start of transfection.

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

Transfection of siRNA (Protocol for initial optimization in a 24-well plate)

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

1. In a 24-well tissue culture plate, seed 0.1 – 1.0 x 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.
   Note: Numbers of cells to seed depend on cell type and size. Optimization may be necessary (determination of growth curve). Maintain same seeding conditions between experiments.
2. The stock solutions of the genetic material 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 other 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:

Tube	R1	R2	R3	R4
Serum-free medium or 1xPBS	30 µl	30 µl	30 µl	30 µl
siRNA	0.1 µg (~ 7.5 pmol)	0.2 µg (~ 15 pmol)	0.5 µg (~ 40 pmol)	2 µg (~ 150 pmol)

Tube	M1	M2	M3	M4
Serum-free medium or 1xPBS	40 µl	40 µl	40 µl	40 µl
PRO-XpressFect	0.5 µl	1 µl	2.5 µl	10 µl

4. Mix the solutions gently by carefully pipetting one time.
5. Combine the solutions as follows:
   R1 + M1
   R2 + M2
   R3 + M3
   R4 + M4
   Mix gently by pipetting up and down once then incubate at room temperature for 15 – 20 minutes.
   Note: The siRNA solutions (R tubes) must be added to the transfection reagent solutions (M tubes). Do not add the transfection reagent solutions to the siRNA solutions.

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

6. Immediately after incubation add the siRNA-lipid complexes dropwise to the cells and **swirl the wells with extreme care** to ensure distribution. Incubate at 37°C in a CO_2 incubator.
7. Depending on cell type, siRNA, stability of the 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 replace with fresh medium.
   Note: 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, replace the complex-containing medium with serum-containing medium 3 – 6 hours after the start of transfection.

Co-Transfection Experiments (siRNA – DNA)

PRO-XpressFect 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 the following recommendations:

1. Plate cells are as those described for transfection of plasmid DNA.
2. Maintain the same *total* nucleic acid to lipid ration as that used for siRNA alone. If it is necessary to increase the total amount of nucleic acid, increase the amount of PRO-XpressFect in proportion to the total amount (µg) of nucleic acid.
3. Always use a volume of PRO-XpressFect (µl) that is at least double the total final mass of nucleic acid (µg).

Optimization

Critical Optimization Parameters

Ratio of DNA or RNA to PRO-XpressFect

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

Optimal ratio of nucleic acid to PRO-XpressFect and concentration of nucleic acid-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 PRO-XpressFect have shown superior results if transfection is performed in the presence of serum. Special cell lines may show different behavior.

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

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 PRO-XpressFect and nucleic acid. Serum may inhibit complex formation. Once the complex is formed, contact with serum is permitted.

Optimal ratio of DNA or RNA to PRO-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 1X PBS in nucleic acid-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 $Na_2HPO_4 \bullet 2\ H_2O$

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.

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

Optimization Protocol

For optimizing purposes, use reporter gene plasmids such as pCMVßGal, pND2Lux, pEGFP etc.

1. Follow the working instructions, varying the amount of PRO-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. PRO-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 PRO-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 tissue culture plate, seed 1.0 – 4.0 x 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 CO_2 incubator until the growing area is 90 – 200% covered. The time required will vary among cell types but will usually take 18 – 24 hours.
3. The stock solutions of the genetic material and PRO-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 a cell culture grade 96-well plate. Then add the following:
   D1 – D4 1 µl, 2 µl, 4 µl, 6 µl PRO-XpressFect
   E1 – E4 2 µl, 4 µl, 8 µl, 12 µl PRO-XpressFect
   F1 – F4 4 µl, 8 µl, 12 µl, 16 µl PRO-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 gently by pipetting up and down once** (shear stress can destroy the developing complex). Incubate at room temperature for 15 – 20 minutes.

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

Note: The DNA or RNA solution from step 4 MUST be added to the PRO-XpressFect solution from step 5. DO NOT add the PRO-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 CO_2 incubator.
   Note: If toxicity is a problem because of highly sensitive cells, remove the transfection mixture after 3 – 6 and replace with complete 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.

Up and Downscale

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

1. In a majority of the cases, the optimum ratio range of nucleic acid (µg) to PRO-XpressFect (µl) is between 1:2 and 1:7. For siRNA applications, use the siRNA specific optimization protocol.
2. Adapt the ratio of nucleic acid:lipid to your size of wells by the appropriate proportional factor.
3. Adsorption processes of the vessel material with the agents used necessitate optimization of the amount of lipoplex and the nucleic acid-lipid ratio for each change to a significantly different format.

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]	0.04–0.3 (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)
PRO-XpressFect - 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 or RNA [µl]	15 - 30	30	50	100	300	700
Dilution volume of PRO-XpressFect [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

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