

PROTEOfectene®

The high efficiency Proteofection Reagent
for Mammalian Cells

For ordering information, SDS, publications and application notes see www.biont.com

Product	Order No.	Size
PROTEOfectene®	E010-0.1	PROTEOfectene® 100 µl R-Phycoerythrin 100 µl
PROTEOfectene®	E010-0.25	PROTEOfectene® 250 µl R-Phycoerythrin 100 µl

Shipping: At room temperature

Storage: PROTEOfectene® 4°C (**do not freeze**)
R-Phycoerythrin 4°C (**do not freeze**)

Stability: Best before: see label

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

Description

The delivery of proteins into living cells represents an alternative to nucleic acid transfection and a powerful strategy for functional studies. This reagent opens new fields of investigation in the rising field of proteomics to elucidate complex molecular mechanisms. The proteins delivered into cells with PROTEOfectene® retain their structure and function. There is no need for covalent linking: just mix PROTEOfectene® with your protein of interest. PROTEOfectene® is a lipid-based formulation which forms non-covalent complexes with proteins. These complexes are internalized by cells and the proteins are released into the cytoplasm.

Index

1. General Information	3
1.1 Specifications	3
1.2 Quality Control	3
1.3 Explanatory Remarks	3
Important criteria for efficient protein delivery into cells	3
Important Parameter: Protein purity	3
2. Standard Protocol	4
2.1 General Considerations	4
2.2 Preparation of the Cells	4
Adherent cells	4
Suspension cells	4
2.3 Formation of the Proteoplex	5
3. Optimization Protocol	6
3.1 Protein : Lipid Ratio	6
3.2 Amount of Protein	6
3.3 Other Parameters	7
Cell density	7
Dilution buffer of the protein	7
Incubation time	7
Presence/absence of serum	7
Transfection volume	7
4. Troubleshooting	8
4.1 Positive control	8
4.2 Low delivery efficiency	9
Protein purity	9
Cell density	9
Cell condition	9
Medium used for preparing the proteoplex	9
Old Proteoplexes	9
PROTEOfectene [®] temperature	9
PROTEOfectene [®] storage	9
4.3 Cellular toxicity	10
Concentration of proteoplex is too high	10
Unhealthy cells	10
Protein is cytotoxic	10
Incubation time	10
Protein quality	10
Key protein delivered	10
5. Miscellaneous	11
5.1 Important Information	11
5.2 Warranty	11

1. General Information

1.1 Specifications

Application	Protein delivery into living mammalian cells
Sterility	tested
Assays	100 µl: max. 100 (24-well plate); max. 20 (6-well plate)
Cell culture	tested
Storage	4°C

R-Phycoerythrin (positive control, 100 µg/ml) is a fluorescent protein with a molecular weight of approx. 240 kDa.

Excitation wave lengths: 480, 545 and 565 nm. Emission wavelength: 578 nm (visible red).

1.2 Quality Control

To assure the performance of each lot of PROTEOfectene[®], each component is tested using rigorous standards. The following assays are conducted *in vitro* to ensure the functioning, quality and activity of each kit component.

Purity: Silica Gel TLC assay

Sterility: Thioglycolate assay

Biological Activity: Delivery of R-Phycoerythrin in NIH3T3 cells monitored by cytofluorimetry and fluorescence microscopy.

1.3 Explanatory Remarks

Important criteria for efficient protein delivery into cells

Proteins differ from one another in term of size, structure, composition and bio-physical properties. Unlike different nucleic acids which have very similar bio-physical properties, the association of proteins with PROTEOfectene[®] is highly variable. Thus, optimal delivery conditions for one particular protein cannot be transferred to another type of protein.

Due to their specific properties some proteins might not be efficiently delivered with PROTEOfectene[®]. For instance, highly alkaline proteins that have an elevated isoelectric point are very difficult to deliver into cells (see troubleshooting chapter 4.2)

However, there are no rules to determine whether a specific protein can be delivered or not. We thus encourage you to trial evaluation of PROTEOfectene[®] with your protein of interest. Delivery efficiency can also vary from one cell line to another.

Important Parameter: Protein purity

Any impurities, contaminants or additives present with your protein of interest may affect delivery efficiency. Consequently, use as pure a recombinant protein as possible.

Stabilizers such as detergents can inhibit the delivery if present excessively in comparison to the protein of interest. Stabilizers such as glycerol or other similar additives do not interfere with the protein delivery.

Preservatives such as sodium azide could hypothetically lead to cytotoxicity if present in high concentrations. It can be removed by dialysis if necessary.

2. Standard Protocol

2.1 General Considerations

The instructions given below represent a standard protocol that was applied successfully on a variety of cells. PROTEOfectene® has been extensively tested and optimized in order to provide you with a very simple, straightforward and efficient procedure. It is best to start by following the standard protocol as a general guideline. Optimal conditions and parameters do vary from protein to protein and from cell line to cell line and have to be found for each new setup, as described in Chapter 3 Optimization Protocol.

R-Phycoerythrin (100 µg/ml) is provided with PROTEOfectene® as a positive control. Use it with a protein:lipid ratio of 1:2 (for 1 µg of protein 2 µl of PROTEOfectene® are needed). This control protein is provided to help you set up your experiment and should be used for each new cell line with which you experiment (see chapter 4.1).

Please note that the purity of the protein and the presence or absence of additives and contaminants has a high impact on the delivery efficiency.

2.2 Preparation of the Cells

Adherent cells

It is recommended to seed or plate the cells the day before the protein delivery experiment. The suitable cell density will depend on the growth rate and the condition of the cells. Cells should be 50–70% confluent (percentage of growth surface covered with cells) at the time of experiment.

Suspension cells

For fast-growing cells, split the cells the day before the protein delivery experiment at a density of $2 - 5 \times 10^5$ cells/ml, in order to keep them in excellent condition.

Table 1: Number of cells to seed for various cell culture formats

Culture vessel	Number of adherent cells	Number of suspension cells	Cell overlay volume
96 well	$0.05 - 0.15 \times 10^5$	$0.5 - 1 \times 10^5$	100 µl
24 well	$0.5 - 1 \times 10^5$	$1.5 - 5 \times 10^5$	400 µl
12 well	$1 - 2 \times 10^5$	$2.5 - 10 \times 10^5$	900 µl
6 well	$2.5 - 5 \times 10^5$	$5 - 20 \times 10^5$	1.8 ml
60 mm dish	$5 - 10 \times 10^5$	$1 - 5 \times 10^6$	3.8 ml
90 – 100 mm dish	$12 - 30 \times 10^5$	$2.5 - 10 \times 10^6$	7.6 ml
T-75 flask	$15 - 40 \times 10^5$	$5 - 15 \times 10^6$	9.6 ml

2.3 Formation of the Proteoplex

1. **Dilute the protein** in 1x PBS at **100 µg/ml**.

While a small concentration of glycerol (1–5%) in the protein solution is acceptable, BSA can completely inhibit the protein delivery.

2. **Pipet the protein** (100 µg/ml) into a microtube, according to the Table 2.

Do not dilute PROTEOfectene®. If pipetting of small quantities is required, prepare a greater amount of proteoplex (protein–PROTEOfectene®–complex).

3. **Add PROTEOfectene®** to the microtube containing the protein, according to Table 2. Mix by gently pipetting up and down several times.
4. **Incubate** for 10–15 min at room temperature.
5. **Add serum-free medium** to the proteoplex (see dilution volume in Table 2) and disperse immediately onto the cells growing in their regular culture medium (with serum). For suspension cells, mix complexes with the cell solution by pipetting the medium up and down (3–4 times) to ensure homogenous distribution of the mixture.
6. **Incubate the cells** under standard conditions (for example at 37°C in a CO₂ atmosphere) until evaluation of the protein delivery efficiency (3–48 h). Incubation time see chapter 3.3 Other Parameters.

Table 2: Standard amount of protein and PROTEOfectene®, dilution volume and total volume per well/dish for various cell culture formats

Culture vessel	Protein [µg]	PROTEOfectene® [µl]	Dilution Volume [µl]	Total Medium Volume
96 well	0.4	0.8	20	120 µl
24 well	1	2	100	500 µl
12 well	2	4	100	1 ml
6 well	5	10	200	2 ml
60 mm dish	10	20	200	4 ml
90 – 100 mm dish	30	60	400	8 ml
T-75 flask	35	70	400	10 ml

3. Optimization Protocol

3.1 Protein : Lipid Ratio

Start by optimizing the protein:lipid ratio for the used protein and particular cell type (Table 3). To do this, use a fixed amount of protein and vary the protein:lipid ratio from 0.5:1 to 1:5, starting at the protein amount given in the standard protocol (Table 2). For instance, from 0.5 to 5 µl of PROTEOfectene[®] reagent in a 24-well plate with 1 µg of protein.

3.2 Amount of Protein

Then increase the amount of protein to be delivered while maintaining the previously determined ratio of protein to PROTEOfectene[®].

Table 3: Optimization of protein amount and volume of PROTEOfectene[®] reagent

Culture vessel	Protein [µg]	PROTEOfectene [®] [µl]	Dilution Volume [µl]	Total Medium Volume
96 well	0.2 – 0.5	0.2 – 1	20	120 µl
24 well	0.5 – 2	0.5 – 5	100	500 µl
12 well	1 – 4	1 – 10	100	1 ml
6 well	2.5 – 10	2.5 – 25	200	2 ml
60 mm dish	5 – 20	5 – 50	200	4 ml
90 – 100 mm dish	15 – 60	15 – 120	400	8 ml
T-75 flask	20 – 80	20 – 160	400	10 ml

3.3 Other Parameters

After having identified the optimal protein:lipid ratio and protein amount, you may continue to optimize if desired by varying other parameters, as listed below.

Cell density

Best results are reached when cells are 50–70% confluent (percentage of growth surface covered with cells) at the delivery time.

Dilution buffer of the protein

1x PBS is recommended but other buffers (e.g. TRIS, HEPES, HBS) may be more appropriate depending on proteins.

Incubation time

The optimal space of time between delivery and assay varies with cells, type of protein, kinetics of biological function, etc. As assays are type-dependent it is recommended to perform a time-course experiment to set up the optimal incubation time which will vary with – for example – protein activity or the half-life of the protein. The delivery efficiency can be determined after 4-96 h.

Presence/absence of serum

PROTEOfectene® can be used on cells in absence of serum. In this case, replace the complete culture medium with serum-free medium. This procedure may be more efficient at delivering certain proteins in some cells. HEPES, HBS or TRIS buffer can be used instead of PBS to prepare the protein solution in this case. Add some serum-containing medium after 3–4 h, if further incubation time is needed.

Transfection volume

To increase delivery efficiency, the transfection volume (total medium volume in Table 2) can be reduced for the first 4-24 h.

4. Troubleshooting

4.1 Positive control

If the evaluation of the test shows no protein delivery, a positive control within the test can indicate possible causes for the lack of delivery.

If the positive control shows protein delivery into cells, but the sample with your protein does not, it is probable that cell condition and density as well as general handling were in order. The error search should primarily focus on parameters affecting the proteoplex formation (protein:reagent-ratio; type and pH of the buffer; type, charge and purity of the protein).

If both, the positive control and the sample, show no protein delivery, further experiments should be conducted using the positive control only, before continuing tests with your own protein. R-Phycoerythrin has been delivered into many different cells, the probability of a successful transport is therefore high. The error search should therefore initially be focused on condition, health and type of the used cells.

If cytotoxicity is a problem, the positive control enables the user to determine whether the delivered protein is influencing cell viability.

4.2 Low delivery efficiency

Protein purity

Make sure that the recombinant protein is highly pure and devoid of additives such as BSA or detergents.

Cell density

A non-optimal cell density at the time of protein delivery can lead to insufficient uptake. The optimal confluence ranges from 50-70%.

Cell condition

Cells that have been in culture for a long time (> 8 weeks) may become resistant to the delivery. Use freshly thawed cells that have been passaged at least once.

Cells should be healthy and in their exponential growth phase during the assay. The presence of contaminants (e.g. mycoplasma) diminishes the delivery efficiency considerably.

Medium used for preparing the proteoplex

Change the protein dilution buffer or the pH to improve the delivery. Highly alkaline proteins are difficult to deliver due to the presence of positive charges. This can be compensated in part by the hydrophobic properties of the protein. The charge of the protein can be modified by means of the pH.

Old Proteoplexes

The Proteoplexes have to be freshly prepared every time. Complexes prepared and stored for more than 1 h aggregate, which leads to delivery of inactive clusters. Depending on the protein, reduce this time to avoid the aggregation which may occur during the complex formation.

PROTEOfectene[®] temperature

The protein solutions and the reagent should have room temperature and should be vortexed prior to use.

PROTEOfectene[®] storage

Delivery efficiency can decrease if PROTEOfectene[®] is kept at room temperature for more than one week.

4.3 Cellular toxicity

Concentration of proteoplex is too high

Decrease the amount of protein complex added to the cells by lowering the amount of protein while keeping the protein:lipid ratio constant. Complex aggregation can cause toxicity; prepare complexes freshly and adjust the ratio as outlined in chapter 3.

Unhealthy cells

- Check cells for contamination (e.g. for mycoplasma).
- Use a new batch of cells, passaged at least once.
- Ensure optimal culture medium conditions (e.g. pH or type of medium used).
- Make sure cells are not too confluent or cell density is not too low. Cells should be in the exponential growth phase.

Protein is cytotoxic

Use suitable controls such as untreated cells and a positive control (with R-Phycoerythrin).

Incubation time

Reduce the incubation time of complexes with the cells. Delivery medium can be replaced by fresh medium after 3-24 h if necessary.

Protein quality

Use highly pure protein as impurities can lead to cell death.

Key protein delivered

The delivered protein itself can have an impact on cell vitality if it influences key points of the cells' metabolism.

5. Miscellaneous

5.1 Important Information

This reagent is developed and sold for research purposes and *in vitro* use only. It is not intended for human or animal therapeutic or diagnostic purposes.

PROTEOfectene® is a registered trademark of Biontex Laboratories GmbH.

5.2 Warranty

Biontex guarantees the performance of this product until the date of expiry printed on the label when stored and used in accordance with the information given in this manual. If you are not satisfied with the performance of the product please contact Biontex Laboratories GmbH.

*Biont*ex Laboratories GmbH
Landsberger Straße 234
im MGH

80687 München/Laim
Germany

Tel.: +49 (0)89 3247995-0
Fax: +49 (0)89 3247995-2
E-Mail: [ex.com](mailto:contact@biontex.com
Internet: <a href=)