PROTEOfectene® AB

The high efficiency antibody Proteofection Reagent for Mammalian Cells

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

Product	Order No.	Size
PROTEOfectene® AB	E020–0.1	PROTEOfectene® AB 100 µl FITC–IgG 100µl
PROTEOfectene® AB	E020–0.25	PROTEOfectene® AB 250 µl FITC–IgG 100µl

Shipping: At room temperature

Storage: PROTEOfectene® AB 4°C (**do not freeze**)
FITC–IgG ≤ -15°C

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 antibodies 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 antibodies delivered into cells with PROTEOfectene® AB retain their structure and function. There is no need for covalent linking: just mix PROTEOfectene® AB with your antibody of interest. PROTEOfectene® AB is a lipid-based formulation which forms non-covalent complexes with antibodies. These complexes are internalized by cells and the antibodies are released into the cytoplasm.

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1. General Information

1.1 Specifications

Application	Antibody delivery into living mammalian cells
Sterility	tested
Assays	100 µl: max. 100 (24-well plate); max. 20 (6-well plate)
Cell culture	tested
Storage	PROTEOfectene® AB: +4°C; FITC-IgG: ≤ -15°C

FITC–labeled IgG (positive control, 100 µg/ml) is a fluorescence–labeled antibody (Immunoglobulin).

Excitation wave length: 488 nm.

Emission wavelength: 520 nm (visible green).

1.2 Quality Control

To assure the performance each batch of PROTEOfectene® AB is tested using rigorous standards. The following assays are conducted:

Purity:	Silica gel TLC assay
Sterility:	Thioglycolate assay
Biological Activity:	Delivery of FITC–labeled IgG into NIH3T3 cells monitored by cytofluorimetry and fluorescence microscopy.

1.3 Important Parameter: Antibody purity

Any impurities, contaminants or additives present in the antibody solution of your antibody of interest may affect delivery efficiency. For this reason, the antibody should be as pure as possible.

BSA in the antibody solution inhibits antibody delivery! 0.1% to 2% BSA are common concentrations in antibody solutions – this means 1 mg/ml to 20 mg/ml. The proportion of BSA greatly exceeds the antibody in these solutions. BSA competes with the antibody during the formation of the antibody–PROTEOfectene® AB–complex and therefore inhibits antibody delivery.

Other stabilizers such as detergents can also inhibit delivery if present in excess in relation to the antibody. Stabilizers such as glycerol or other similar additives do not interfere with the antibody delivery, however.

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

2. Standard Protocol

2.1 General Considerations

The instructions given below represent a standard protocol that was applied successfully on a wide variety of cell types. It is best to start by following the standard protocol as a general guideline. Optimal conditions and parameters do vary from cell line to cell line and have to be found for each new setup, as described in chapter 3.

PROTEOfectene® AB is provided with FITC–IgG (100 µg/ml) as a positive control. Use it with a antibody:reagent ratio of 1:1 – 1:2 (for 1 µg of antibody 1-2 µl of PROTEOfectene® AB are needed). This control antibody 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).

The purity of the antibody and the presence or absence of additives and contaminants has a high impact on the delivery efficiency.

2.2 Preparing the cells

Adherent cells

Seed or plate the cells the day before the antibody 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 antibody delivery experiment at a density of 2 – 5 x 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 x 10^5	0.5 – 1 x 10^5	100 µl
24 well	0.5 – 1 x 10^5	1.5 – 5 x 10^5	400 µl
12 well	1 – 2 x 10^5	2.5 – 10 x 10^5	900 µl
6 well	2.5 – 5 x 10^5	5 – 20 x 10^5	1.8 ml
60 mm dish	5 – 10 x 10^5	1 – 5 x 10^6	3.8 ml
90 – 100 mm dish	12 – 30 x 10^5	2.5 – 10 x 10^6	7.6 ml
T–75 flask	15 – 40 x 10^5	5 – 15 x 10^6	9.6 ml

2.3 Forming of Proteoplex

1. **Dilute the antibody** in 1x PBS to 100 µg/ml.

The presense of glycerol (1-5%) in antibody solution does not interfere with the antibody delivery experiment. BSA completely inhibits the antibody delivery!

2. **Pipet the antibody** (100 µg/ml) into a microtube, according to table 2.
3. **Add PROTEOfectene® AB** to the microtube containing the antibody, according to table 2. Mix by gently pipetting up and down several times.

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

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

Culture vessel	Antibody [µg]	PROTEOfectene® AB [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

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, add the proteoplex to the cell solution and mix by pipetting the medium up and down gently (3–4 times) to ensure a homogenous distribution of the proteoplex.

6. **Incubate the cells** under standard conditions (for example at 37°C in a CO_2 atmosphere) until evaluation of the antibody delivery efficiency (3–48 h).

3. Optimization Protocol

3.1 Antibody : Lipid ratio

Start by optimizing the antibody:reagent ratio for the antibody and particular cell type used (Table 3). To do this, use a fixed amount of antibody and vary the antibody:reagent ratio from 1:0.5 to 1:10 starting at the antibody amount given in the standard protocol (Table 2). That means 0.5 to 10 µl of PROTEOfectene® AB reagent is used in a 24–well plate for 1 µg of antibody.

3.2 Amount of antibody

Then increase the amount of antibody to be delivered while maintaining the previously determined ratio of antibody:reagent-ratio.

Table 3: Optimization of antibody amount and volume of PROTEOfectene® AB reagent

Culture vessel	Antibody [µg]	PROTEOfectene® AB [µl]	Dilution Volume [µl]	Total Medium Volume
96 well	0.2 – 0.5	0.2 – 1	20	120 µl
24 well	0.5 – 3	0.5 – 5	100	500 µl
12 well	1 – 6	1 – 10	100	1 ml
6 well	2.5 – 15	2.5 – 25	200	2 ml
60 mm dish	5 – 30	5 – 50	200	4 ml
90 – 100 mm dish	15 – 75	15 – 120	400	8 ml
T–75 flask	20 – 80	20 – 160	400	10 ml

3.3 Other Parameters

After having identified the optimal antibody:reagent ratio and antibody 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 antibody

1x PBS is recommended, do not use other buffers.

Incubation time

The optimal space of time between delivery and assay varies with the used cells, antibody isotype and biological function. Perform a time–course experiment to set up the optimal incubation time which will vary as binding of the antibody to its target is dependent on the target localization and accessibility as well as the protein turnover rate.

The delivery efficiency can be determined after 4-96 h.

Presence/absence of serum

PROTEOfectene® AB can be used in serum–free medium. In this case, replace the complete culture medium with serum–free medium. This procedure may be more efficient at delivering antibodies in some cells. Add some serum–containing medium after 4 h, if further incubation time is needed.

Transfection volume

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 antibody delivery, a positive control within the test can indicate possible causes for the lack of delivery.

If the positive control shows antibody delivery into cells, but the sample with your antibody 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 (antibody:reagent–ratio; purity of the antibody).

If both, the positive control and the sample, show no antibody delivery, further experiments should be conducted using the positive control only, before continuing tests with your own antibody. FITC–IgG 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 antibody is influencing cell viability.

4.2 Low delivery efficiency

Antibody purity

The antibody solution must not contain BSA!

Make sure that the antibody is highly pure and devoid of additives such as stabilizers or detergents.

Cell density

A non–optimal cell density at the time of antibody delivery can lead to insufficient uptake of the proteoplex. The optimal confluence (percentage of growth area covered by cells) ranges from 50-70%.

Cell condition

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

Cells should be healthy and proliferating well during the assay. The presence of contaminants (e.g. mycoplasma) diminishes the delivery efficiency considerably.

Medium used for preparing the proteoplex

It is vital to use PBS for the formation of the proteoplex. The use of other buffers is not recommended.

Old Proteoplexes

Proteoplexes have to be freshly prepared every time. Proteoplexes prepared and stored for more than 1 h aggregate, which leads to delivery of inactive clusters. Add proteoplexes immediately after their formation.

PROTEOfectene® AB temperature

The antibody solutions and the reagent should be used at room temperature and should each be mixed gently prior to use.

PROTEOfectene® AB storage

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

4.3 Cellular toxicity

Concentration of Proteoplexes is too high

To decrease the amount of Proteoplexes, lower the amount of antibody during complex formation while keeping the antibody:reagent 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 freshly thawed 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 50-70% confluent.

Antibody is cytotoxic

Use suitable controls such as untreated cells and a positive control (with FITC–IgG).

Incubation time

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

Antibody quality

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

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.

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