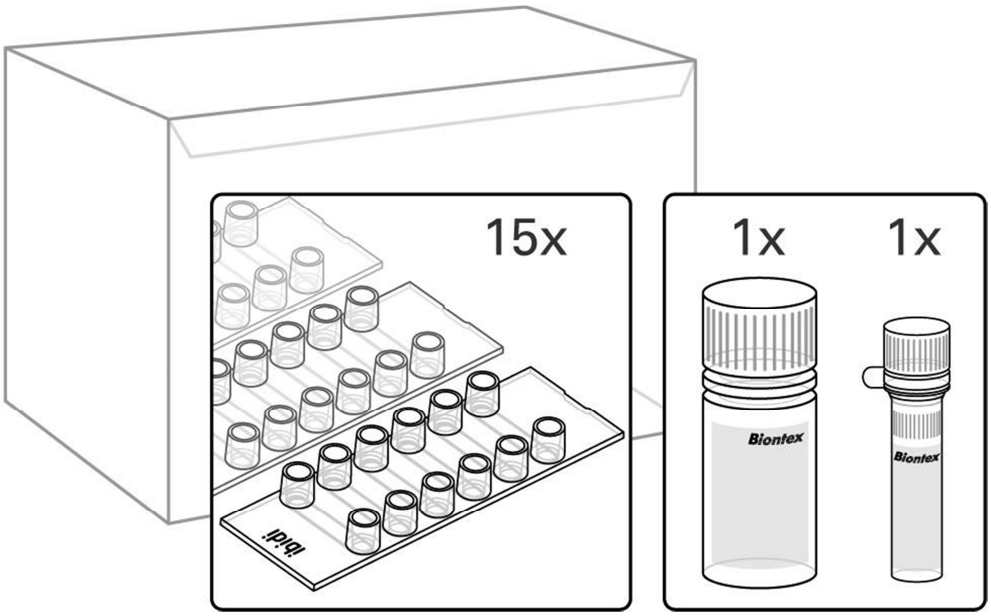
Cell Culture, Proteofection of Antibodies and Microscopy in a Slide

The **μ-Proteofection Kit VI AB** combines the outstanding optical properties of μ–Slides VI from ibidi with the high–efficiency reagent for antibody proteofection PROTEOfectene® AB from Biontex. The kit provides ideal conditions for high–resolution microscopic observation of living cells proteofected with antibodies.

PROTEOfectene® AB complexates antibodies noncovalently by means of electrostatic and hydrophobic interactions, forming a proteoplex which is taken up by cells through endocytosis. With the vast variety of commercially available antibodies, PROTEOfectene® AB opens up new possibilities for the investigation and manipulation of cellular processes of all kinds.

The **μ–Slide VI** is designed for high–resolution microscopy and is optimal both for experiments with living cells and for cell fixation and staining. Thanks to the high optical quality of the material fluorescence microscopy is possible at any wavelength and at maximum resolution.



Contents μ–Proteofection Kit VI AB:		
PROTEOfectene® AB	250 µl	Biontex Laboratories GmbH
FITC-IgG (Positive control; 100 µg/ml in PBS)	100 µl	Biontex Laboratories GmbH
μ–Slide VI, ibiTreat	15 x	ibidi GmbH

Shipping:	At room temperature	
Storage:	PROTEOfectene® AB FITC-IgG μ–Slide VI	4°C; do not freeze ≤ -15°C room temperature
Stability:	Best before: see label	
Use:	Only for research purposes in vitro, not intended for human or animal diagnostic, therapeutic or other clinical uses.	

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1 General Information

1.1 Working with ibidi µ–Slides

1.1.1 Material

The µ–Slides consist of a plastic with highest optical quality. The material exhibits extremely low birefringence and autofluorescence, both similar to that of glass. It is not possible to detach the bottom from the slide. The µ–Slides are not autoclavable since they are temperature stable up to 60°C/140°F only. Please note that gas exchange between the channel and incubator's atmosphere occurs partially through the plastic bottom which should not be covered. Thus, it is recommended to place the µ–Slide on an ibidi µ–Slide rack.

1.1.2 Geometry of the µ–Slide VI

The µ–Slide VI provides standard slide format according to ISO 8037/1. The lateral adapter to adapter distance of 9 mm (like 96 well plates) allows using multichannel pipettes.

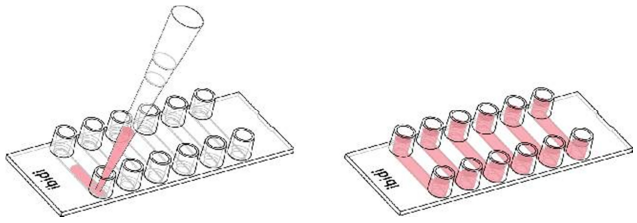
Geometry	
Number of channels	6
Channel volume	30 µl
Channel length	17 mm
Channel width	3.8 mm
Channel height	0.4 mm
Adapters	female Luer
Volume per reservoir	60 µl
Growth area	0.6 cm^2 je Kanal
Coating area using 30 µl	1.2 cm^2 je Kanal
Bottom matches coverslip	No. 1.5

1.1.3 Preparation for Cell Microscopy

To analyze your cells no special preparations are necessary. Cells can be observed live or fixed directly in the µ–Slide on an inverted microscope. You can use any fixative of your choice. The µ–Slide material is compatible with a variety of chemicals, e.g. Acetone or Methanol. Further specifications can be found at www.ibidi.com. Due to the thin bottom of only 180 µm, high resolution microscopy is possible.

1.1.4 Filling of the Slides

Apply 30 µl of cell suspension per channel of the µ–Slide–VI with a pipet. To avoid air bubbles put the pipet tip right on the channel inlet and point at the channel as shown in the picture below.



After cell attachment (approx. 4 – 6 hours) fill the reservoirs with 60 µl medium each.

1.1.5 Immersion Oil

When using oil immersion objectives, only the immersion oils specified in the table may be used. The use of different oil can lead to damages of the objective.

Company	Product	Ordering number
Cargille	type DF, Code: 1261	(Cargille) 16242
Cargille	type DF37, Code: 0383	(Cargille) 16239
Cargille	Series AAA, n = 1.330	(Cargille)
Cargille	Series AAA, n = 1.335	(Cargille)
ibidi	immersion oil	(ibidi) 50101
Olympus	8 ml	(Olympus) 035520
Zeiss	518 F	(Zeiss) 444960

Tip:

The day before seeding the cells we recommend to place the cell medium and the µ–Slide into the incubator for equilibration. This will prevent the liquid inside the channel from emerging air bubbles over the incubation time.

Trapped air bubbles can be removed from the channel by inclining the µ–Slide and knocking at one edge.

1.2 Working with PROTEOfectene® AB

1.2.1 Specification

Application	Delivery antibodies into living mammalian cells in µ–Slides VI
Sterility	tested
Cell culture	tested
Stability	1 year
Storage	4 °C

FITC–labeled IgG (positive control) is a fluorescence labeled antibody (immunoglobulin). Excitation maximum: 488 nm. Emission maximum: 520 nm (visible green).

1.2.2 Quality Control

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

Purity:	Silica Gel TLC assay
Sterility:	Thioglycolate assay
Biological Activity:	Delivery of FITC–IgG in NIH3T3 cells monitored by cytofluorometry and fluorescence microscopy.

1.2.3 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 proteoplex 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 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 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).

Note: The purity of the protein and the presence or absence of additives and contaminants have a high impact on the delivery efficiency.

The following instructions refer to one channel of a µ–Slide VI.

2.2 Preparing the Reagents

Prior to antibody delivery (proteofection), bring PROTEOfectene® AB and the antibody to room temperature. Mix each solution gently.

2.3 Preparing the Cells

Seed the cells on 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.

1. Prepare a cell suspension with a concentration of $3 – 4 \cdot 10^5$ cells/ml in complete culture medium.
2. Fill 30 µl of the cell suspension into one channel of the µ–Slide VI.
3. When the cells have adhered (approx. 2 – 6 h after seeding) fill each reservoir of the channel with 60 µl of complete culture medium.

2.4 Preparing the Proteoplex

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

Note: BSA completely inhibits the antibody delivery!

5. Pipet 0.4 µg antibody (4 µl; 100 µg/ml) into a microtube.
6. Add 0.8 µl PROTEOfectene® AB. Mix by gently pipeting up and down the whole volume.

Note: Do not dilute PROTEOfectene® AB. If pipeting of small quantities is required, prepare a greater amount of proteoplex

7. Incubate for 10 – 15 min at room temperature.
8. Meanwhile remove the culture medium from the reservoirs of the cell–filled channel. Keep the channel itself filled.
9. After the incubation period, add 30 µl of complete cell culture medium to the microtube containing the proteoplex.

2.5 Proteofection

10. Now swap the culture medium present in the channel with the proteoplex solution in the microtube. To do so, tilt the slide approximately 45 degree over its longitudinal axis and pipet 30 µl of the proteoplex from the microtube into the upper reservoir. The proteoplex now displaces the culture medium from the channel. The geometrical conditions given within the channel prevent the two solutions from mixing significantly. Remove the 30 µl of the displaced culture medium from the lower reservoir.
11. Incubate the slide under standard conditions for the cells.
12. 6 h later, fill both reservoirs with 60 µl of complete culture medium, respectively.
13. The culture medium has to be changed daily in assays of a total duration longer than two days.

3 Optimization Protocol

3.1 Antibody:Reagent Ratio

Start by optimizing the antibody:reagent ratio for the particular cell type used. To do this, take the given starting-amount of 0.4 µg of antibody and vary the antibody:reagent ratio from 1:0.5 to 1:5. That means for 0.4 µg of antibody 0.2 µl to 2 µl of PROTEOfectene® AB are used.

3.2 Amount of Antibody

Then increase the amount of antibody to be delivered while maintaining the previously determined ratio of antibody to PROTEOfectene® AB. 0.1 µg to 0.8 µg per channel are antibody amounts that have worked well in the past. However, these values can be exceeded or undershot in individual cases.

3.3 Alternative Protocol

If the amount of antibody delivered into cells is low despite having optimized the antibody:reagent-ratio and the amount of antibody, the period of time between seeding the cells and adding the proteoplex can be shortened. This can – depending on the used cell line – increase the amount of antibody delivered:
Seed the cells; incubate for 6 h; add proteoplex; incubate overnight; fill reservoirs with complete cell culture medium; incubate until evaluation.

3.4 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.

3.4.1 Cell Density

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

3.4.2 Dilution Buffer of the Antibody

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

3.4.3 Incubation Time

The optimal space of time between delivery and evaluation varies with the used cells, antibody isotype, biological function, etc.

Perform a time-course experiment to set up the optimal incubation time which will vary as binding of 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 6 – 96 h.

3.4.4 Presence/Absence of Serum

PROTEOfectene® AB can be used on cells 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 6 h, if further incubation time is needed.

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

4.2.1 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.

4.2.2 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%.

4.2.3 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.

4.2.4 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.

4.2.5 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.

4.2.6 PROTEOfectene® AB Temperature

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

4.2.7 PROTEOfectene® AB Storage

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

4.3 Cellular Toxicity

4.3.1 Concentration of Proteoplex is too High

To decrease the amount of proteoplex, 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.

4.3.2 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.

4.3.3 Antibody is Cytotoxic

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

4.3.4 Incubation Time

Reduce the incubation time of cells with complexes. Delivery medium can be replaced by fresh medium after 6 – 24 h if necessary.

4.3.5 Antibody Quality

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

5 Miscellaneous

5.1 Important Information

µ–Proteofection Kit VI AB is developed and sold for research purposes and in–vitro use only. It is not intended for human therapeutic or diagnostic purposes.

5.2 Warranty

Biontex guarantees the performance of PROTEOfectene® AB, when used in accordance with the information given in this publication, to the expiration date printed on the vial. If you are not satisfied with the performance of PROTEOfectene® AB (Biontex) or the µ–Slides (ibidi) contact us directly or via one of the authorized distributors.

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