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ProteoAB-XpressFect

Catalog Number	Size
ABPXF10-1	ProteoAB-XpressFect 100 µl FITC IgG 100 µL
ABPXF10-25	ProteoAB-XpressFect 250 µl FITC IgG 100 µL

Description: The delivery of antibodies into living cells represents an alternative to nucleic acid transfection and a powerful strategy for functional studies. The ProteoAB-XpressFect reagent opens new fields of investigation in the rising field of proteomics to elucidate complex molecular mechanisms. The antibodies delivered into cells with ProteoAB-XpressFect retain their structure and function. There is no need for covalent linking, simply mix ProteoAB-XpressFect with your antibody of interest. ProteoAB-XpressFect 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.

Application: Antibody delivery into living mammalian cells

Assays: 100 µl: 24-well plate, 100 maximum; 6-well plate, 20 maximum

Shipping: Shipped at room temperature.

Storage: +4°C **(Do not freeze)**

Stability: See label for expiration date.

Notes: The FITC-labeled IgG positive control (100 µg/ml) is a fluorescence – labeled antibody. The excitation wavelength is 488 nm and the emission wavelength is 520 nm (visible green).

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

Explanatory Remarks

Antibody Purity

Any impurities, contaminants or additives present in the antibody solution of the antibody of interest may affect delivery efficiency. The antibody must be as pure as possible.

BSA in the antibody solution inhibits antibody delivery. 0.1% to 2% (1 mg/ml to 20 mg/ml) BSA are common concentrations in antibody solutions. The proportion of BSA greatly exceeds the antibody in these solutions. BSA competes with the antibody during the formation of the antibody – ProteoAB-XpressFect complex and therefore inhibits antibody delivery.

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

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

Protocol

The instructions below represent a standard protocol that was applied successfully on a variety of cells. ProteoAB-XpressFect has been extensively tested and optimized to provide the user with a simple, straightforward and efficient procedure. It is best to start by following the standard protocol as a general guideline. Optimal conditions and parameters vary from cell line to cell line and have been found for each new setup, as described in the Optimization Protocol section.

ProteoAB-XpressFect is provided with FITC-IgG (100 µg/ml) as a positive control. Use it with an antibody:reagent ratio of 1:1 – 1:2 (for 1 µg of antibody 1 -2 µl of ProteoAB-XpressFect 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 refer to Troubleshooting section for more information.

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

Cell Preparation

Adherent Cells

It is recommended to 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 the 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 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

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Formation of the Proteoplex

1. Dilute the antibody in 1 x PBS at 100 µg/ml.
   Note: The presence of glycerol (1 – 5%) in antibody solution does not interfere with the antibody experiment. BSA completely inhibits the antibody delivery.
2. Pipet the antibody (100 µg/ml) into a microtube, refer to Table 2 below.
3. Add ProteoAB-XpressFect to the microtube containing antibody, refer to Table 2 below. Mix by pipetting up and down several times.
   Note: Do not dilute the ProteoAB-XpressFect. If pipetting of small quantities is required prepare a greater amount of proteoplex (antibody + ProteoAB-XpressFect).
4. Incubate for 10 – 15 minutes at room temperature.
5. Add serum free medium to the proteoplex (see Dilution Volume column 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 homogeneous distribution of the mixture.
6. Incubate the cells under standard conditions (e.g. 37˚C in CO_2 atmosphere) for 3 – 48 hours, depending on when antibody delivery efficiency is to be evaluated. See Other Parameters section for incubation times.

Table 2. Standard amount of antibody and ProteoAB-XpressFect, dilution volume, and total volume per well/dish for various cell culture formats.

Culture Vessel	Antibody (µg)	ProteoAB-XpressFect (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

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Optimization Protocol

Antibody : Lipid Ratio

Start by optimizing the antibody : lipid ratio for the used antibody and particular cell type, refer to Table 3 below. To do this, use a fixed amount of antibody and vary the antibody : lipid ratio from 0.5:1 to 1:10, starting at the antibody amount given in the standard protocol in Table 2. For example, from 0.5 to 10 µl of ProteoAB-XpressFect reagent in a 24 – well plate with 1 µg of antibody.

Then, increase the amount of antibody to be delivered while maintaining the previously determined ratio of antibody to ProteoAB-XpressFect.

Table 3. Optimization of antibody amount and volume of Proteo-XpressFect reagent.

Culture Vessel	Antibody (µg)	ProteoAB-XpressFect (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

Other Parameters

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

Cell density

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

Dilution buffer of the antibody

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

Incubation time

The optimal space of time between delivery and assay varies with cells, antibody isotype and biological function, etc. 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 hours.

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Presence/absence of serum

ProteoAB-XpressFect 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 hours 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 hours.

Troubleshooting

Positive Control

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

If the positive control shows antibody delivery into cells, but the sample with your antibody does not, it is probable the 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 now 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 therefor the probability of successful transport is high. The error search should be initially focused on condition, health and type of cells used.

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

Low delivery efficiency

Antibody purity

The antibody solution must not contain BSA.

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

Cell density

A non – optimal cell density at the time of antibody delivery can lead to insufficient uptake. The optimal confluency 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) diminished the delivery efficiency considerably.

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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

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

ProteoAB-XpressFect temperature

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

ProteoAB-XpressFect storage

Delivery efficiency can decrease if ProteoAB-XpressFect is kept at room temperature for more than one week.

Cellular toxicity

Concentration of the proteoplex is too high

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 ration as outline in the Optimization Protocol.

Unhealthy cells

- Check cells for contamination (e.g. Mycoplasma)
- Use a new batch of cells, passaged at least once
- Ensure optimal culture medium conditions (e.g. pH, 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.

Antibody is cytotoxic

Use suitable controls such as untreated cells and a positive control with FITC - IgG.

Incubation time

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

Antibody quality

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