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Proteo-XpressFect

Catalog Number	Size
PXF10-1	Proteo-XpressFect 100 µl R-Phycoerythrin 100 µL
PXF10-25	Proteo-XpressFect 250 µl R-Phycoerythrin 100 µL

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

Application: Protein 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.

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

Explanatory Remarks

Criteria for efficient protein delivery into cells

Proteins differ from one another in terms of size, structure, composition and bio-physical properties. Unlike different nucleic acids which have very similar bio-physical properties, the association of proteins with Proteo-XpressFect is highly variable. Therefore, 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 Proteo-XpressFect. For example, highly alkaline proteins that have an elevated isoelectric point are very difficult to deliver into cells (see Troubleshooting section for more information).

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There are no standards to determine whether a specific protein can be delivered or not. We encourage the user to trail and evaluate Proteo-XpressFect with the protein of interest. Delivery efficiency can also vary from one cell line to another.

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.

Protocol

The instructions below represent a standard protocol that was applied successfully on a variety of cells. Proteo-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 protein to protein and from cell line and have been found for each new setup, as described in the Optimization Protocol section.

R-Phycoerythrin (100 µg/ml) is provided with Proteo-XpressFect as a positive control. Use it with a protein : lipid ration of 1:2 (1 µg protein : 2 µl Proteo-XpressFect). This control protein is provided to help the user set up the experiment and should be used for each new cell line (see Troubleshooting section for more information).

> **Note: The purity of the protein 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 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 the experiment.
>
> **Suspension Cells**
>
> For fast growing cells, split the cells the day before the protein delivery experiment at a density of 2 – 5 x 10^5 cells/ml to keep them in excellent condition.

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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

Formation of the Proteoplex

1. Dilute the protein in 1 x PBS at 100 µg/ml.
   Note: 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, refer to Table 2 below.
   Note: Do not dilute the Proteo-XpressFect. If pipetting of small quantities is required prepare a greater amount of proteoplex (protein + Proteo-XpressFect).
3. Add Proteo-XpressFect to the microtube containing protein, refer to Table 2 below. Mix by pipetting up and down several times.
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, mix complexes with the cell solution by pipetting the medium up and down 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 protein delivery efficiency is to be evaluated. See Other Parameters section for incubation times.

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

Culture Vessel	Protein (µg)	Proteo-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

Protein : Lipid Ratio

Start by optimizing the protein : lipid ratio for the used protein and particular cell type, refer to Table 3 below. 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 in Table 2. For example, from 0.5 to 5 µl of Proteo-XpressFect reagent in a 24 – well plate with 1 µg of protein.

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

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

Culture Vessel	Protein (µg)	Proteo-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 protein : lipid ratio and protein 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 protein

1 x PBS is recommended but other buffers (e.g. TRIS, HEPES, HBS) may be more appropriate depending on the 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 protein activity or the half-life of the protein. The delivery efficiency can be determined after 4 – 96 hours.

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Presence/absence of serum

Proteo-XpressFect can be used on cells in the 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 hours 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 hours.

Troubleshooting

Positive Control

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

If the positive control shows protein delivery into cells, but the sample with your protein 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 (protein : reagent ratio, type and pH of buffer, type, charge and purity of the protein).

If both the positive control and the sample show now 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 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 protein is influencing cell viability.

Low delivery efficiency

Protein purity

Make sure the recombinant protein is highly pure and devoid of additives such as BSA or detergents. Impurities can lead to cell death.

Cell density

A non – optimal cell density at the time of protein 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

Change the protein dilution buffer or the pH to improve 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 hour aggregate which leads to delivery of inactive clusters. Depending on the protein, reduce this time to avoid aggregation which may occur during the complex formation.

Proteo-XpressFect temperature

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

Proteo-XpressFect storage

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

Cellular toxicity

Concentration of the 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 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.

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 hours if necessary.

Key protein delivered

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