ProFectin™ Intracellular Protein Delivery Reagent

Catalog Number: R-2000

Feature

1. Simple delivery protocol - ready to use, just mix protein and vortex.
2. Superior cell membrane penetration efficiency within FCS medium.
3. Effective for both adherent and suspension cells.
4. Minimal cytotoxicity, excellent choice for human stem cells manipulations.
5. No medium change after transfection.
6. Highly reproducible results.
7. Products stable at both 4°C and - 20°C.

Component

ProFectin™ Solution: 100 µl, (sterile solution).

Description

ProFectin is a new generation of lipid-based protein delivery reagent optimized for recombinant protein intracellular delivery into mammalian cells to provide following advantages:

- High efficiency in many cell type and formats. At least 3 folds better than the Tat or 11 Arginine Tag mediated protein delivery.
- Lower toxicity than the leading brands PULSin™, excellent efficiency for nuclei protein delivery.
- There is no need to remove protein-ProFectin complexes or change medium after transfection.
- The delivery complexes can be added directly to cells in culture medium, in the presence or absence of serum.
- Affordability: 0.1 ml of ProFectin can typically be used for 50 tests of one well using 1 µg recombinant protein (6 well plate cultures)

The ratio of ProFectin to target protein is fixed at 2 µl ProFectin for 1 µg protein in 50 µl PBS buffer. ProFectin is formulated in a ready-to use format. All users need to do is to add protein and mix. After 15 minutes room temperature incubation, the delivery mix can be added to cells.

Protocol for Transfection of Adherent Cells

1. Transfer 1 µg of target protein into 50 µl PBS buffer, then add 2 µl of ProFectin, mix well.
2. Incubate the mixture at room temperature for 15 minutes.
3. Add the mixture to culture cells.

Table 1 Calculation of maximal protein loading per intracellular delivery

Culture Vessel	**Protein Quantity (µg)** per well	**Medium** per well	**PBS Solution** per mix	**ProFectin** Per well
96 well	0.2 - 0.4	0.12 ml	20 µl	0.2 - 1 µl
24 well	0.5 - 2	0.5 ml	50 µl	0.5 - 5 µl
12 well	1 - 4	1 ml	100 µl	1 – 10 µl
6 well	2.5 - 10	2 ml	200 µl	2.5 – 25 µl
10 cm	15 -60	8 ml	400 µl	15 - 120 µl

The amount of highly purified target protein in delivery mixture is the most important parameter. Not using sufficient amount of protein will result in reduced protein delivery efficiency, but too much protein will generate toxicity. The ratio of ProFectin is optimally fixed at 1 µg protein to 2 µl ProFectin reagent, and should not be changed. Change of medium in 12 hours post delivery mixture incubation may improve cell growth condition, and is recommended. This change of medium after delivery mixture incubation is not absolutely required.

This protocol and above recommendations are optimized for human fibroblast (BJ), 293T and mouse NIH3T3 cells. User should test and find the optimal condition for their cell lines and target proteins.

General Considerations

The instructions given above represent sample protocols that were applied successfully on a variety of cells. Our R&D team has extensively tested and optimized the ProFectin reagent with recombinant Cre protein on pCMC_Loxp_GFP cells as functional assay (which requires recombinant Cre protein efficiently penetrates cell membrane, transport into nuclei and assemble to tetramer to become functional) in order to provide you with the simplest, straightforward and efficient procedure. Therefore, we recommend you to start by following our general protocol as guidelines. Optimal conditions do vary from protein to protein and cell to cell. Note that the purity of the protein and the presence of various additives and contaminants will have a high impact on the protein delivery efficiency. Consequently, we advise you to optimize the delivery parameters in order to achieve the best effects. Consequently, we advise you to optimize the delivery parameters in order to achieve the best effects. Several optimization protocols are provided in the following sections.

Important Parameters

a) Protein Purity

It is clear that any impurities, contaminants or additives present with your protein of interest might affect the delivery efficiency. Consequently, we suggest using a recombinant protein as pure as possible. Stabilizer such as detergents can inhibit the delivery if present in large excess over the protein of interest. Stabilizer such as glycerol, arginine, sucrose or other similar additives does not interfere with the protein delivery experiment. BSA should not exist in protein sample due to the inhibition effect of BSA for ProFectin reagent.

b) Cell Preparation

It is recommended to seed or plate the cells the day prior the protein delivery experiment. The suitable cell density will depend on the growth rate and the condition of the cells. Cells should NOT be more than 80% confluent at the time of experiment.

c) Standard Protein Delivery Procedure

1) Prepare a protein solution. Dilute the protein to be delivered in PBS at 100 µg /ml.

- Do not use tissue culture media for this Step! We recommend using PBS but dependent on the protein used other buffer such as Hepes, HBS or Tris buffer can also be used.
- The presence of a small amount of Glycerol (1-5% in the 100 µg / ml protein solution) does not interfere with protein delivery. In contrast, the presence of BSA can completely inhibit the protein delivery. If BSA is present in your protein sample, we recommend removing it before proceeding with the delivery assay.

2) Transfer 0.4 to 70 µl of ProFectin reagent in a new microtube, according to the Table 1.

- Do not dilute ProFectin reagent.

3) Add PBS diluted protein sample to ProFectin reagent microtube, mix well.

4) Incubate 15 min at room temperature.

5) Transfer 20 to 400 µl (see dilution volume in Table) of serum-free medium to the protein/ProFectin mixture and disperse immediately onto the cells growing in their regular culture medium (with serum).

- Note: incubate protein/ProFectin mixture with serum free medium first for 30min at room temperature, then add normal serum medium for over-night incubation will generate the best protein delivery result.

6) Incubate the cells at normal culture condition for 3 – 48 hrs. Incubation time will dependent on different parameters (functional assay, half life of protein, et al).

Important Note: DyLight 488 labeled recombinant GLRX3 protein can be purchased separately as positive control.

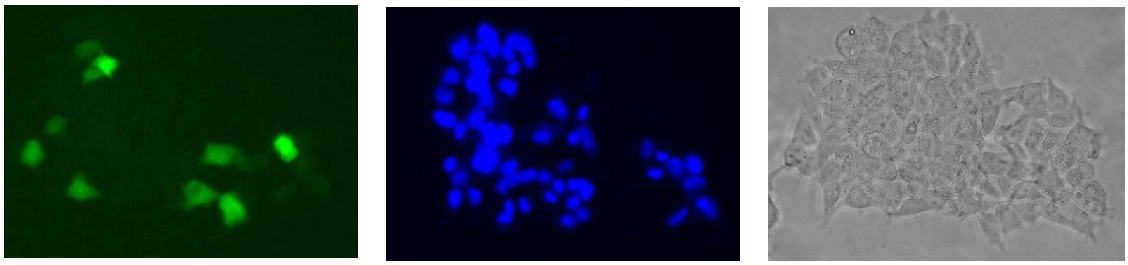
d) Protocol Optimization

In order to get the best out of the ProFectin reagent, several parameters can be optimized:

- Volume of ProFectin reagent,
- Total protein amount per well,
- Dilution buffer of the protein,
- Presence or absence of serum during first 30 min of protein delivery experiment,
- Cell type and seeding density,
- Incubation time.

Storage Condition

Store at 4°C. Stable for 6 months from the date of shipment.



Recombinant Cre protein which was expressed in E.coli and purified with Ni-NTA resin, delivered into 6 well plate with 1 µg protein / 2 µl ProFectin reagent with 293T/pCMV driven-Cre-LoxP-GFP cells in 10% FCS / DMEM medium for 30 min. Data was collected on following day after protein delivery incubation for GFP positive Cells analysis.

Our dedicated and specialized technical support group will be pleased to answer any of your inquiry and to help you with your protein delivery experiments. Please feel free to contact us at Info@ldbiopharma.com for assistance and visit our website: www.LDbiopharma.com to stay informed on the latest breakthrough technologies and updated on our complete product list.