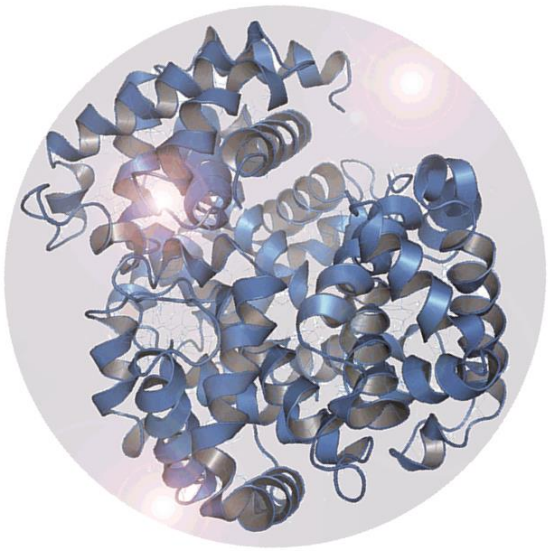


Protein Delivery

Pro-DeliverIN™

Protein Delivery Inside Living Cells

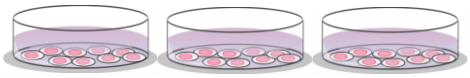
Protocol

OZBIOSCIENCES
The art of delivery systems

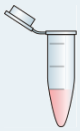
Pro-DeliverIN™ Quick Protocol

To find the ideal conditions, Pro-DeliverIN™ must be tested at ratios **1 µL/µg**, **2 µL/µg** and **2.5 µL/µg** of Pro-DeliverIN / Antibody. For the protein quantity, we suggest **0.4 µg** per well in 96-well, **1 µg** / well in 24-well and **5 µg** / well in 6-well*. Depending on the properties of your protein (size, charge...), the amount used in the test can be doubled (i.e in a 24 well plate, 2 µg of protein instead of 1 µg for 2 / 4 / 5 µL of Pro-DeliverIN).*

1 **Seed cells to be at 70% confluent the day of transfection***



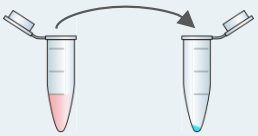
2 **Prepare the protein solution to be delivered at 100 µg/mL in PBS***



3 **Prepare 3 tubes of Pro-DeliverIN™ (with 3 different amounts of reagent)**

96 well plate	24 well plate	6 well plate
0.4µL/0.8µL/1µL in an empty microtube	1µL/2µL/2.5µL in an empty microtube	5µL/10µL/12.5µL in an empty microtube

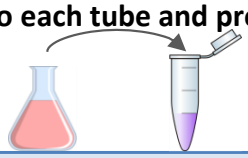
4 **Add the protein solution (step 2) to each tube of Pro-DeliverIN™ (step 3)**



Incubate 10 min at RT

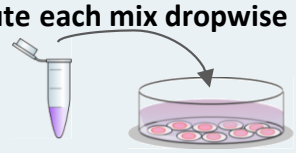
96 well plate	24 well plate	6 well plate
0.4µg (4µL of 100µL/mL solution in PBS) X 3	1µg (10µL of 100µL/mL solution in PBS) X 3	5µg (50µL of 100µL/mL solution in PBS) X 3

5 **Add serum-free medium* to each tube and proceed immediately to the next step**

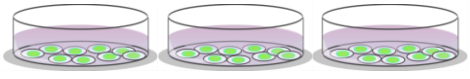


96 well plate	24 well plate	6 well plate
to 20µL	to 100µL	to 200µL

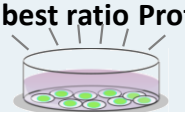
6 **Distribute each mix dropwise onto the cells***



7 **Incubate cells for 3 to 48h at 37°C until evaluation of antibody delivery efficacy**



8 **Choose the best ratio Protein:Pro-DeliverIN™**



These conditions might require some further optimizations depending on your cells, protein, target, etc.

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ Depending on the properties of your protein (size, charge...) **the amount used in the test can be doubled** (*i.e* in a 24 well plate, 2 µg of protein instead of 1 µg for 2 / 4 / 5 µL of Pro-DeliverIN).

- ✓ For cell lines, 24h before transfection seed the cells in a 96-well plate, 24-well plate or 6-well plate in respectively 100 µL, 400 µL and 2 mL of complete culture medium.

- ✓ Allow reagents to reach RT and gently vortex them before forming complexes.

- ✓ **Prepare the protein solution**. Dilute the protein to be delivered in PBS at 100 µg / mL.
  a. ***Do not use culture media for this step!*** We recommend using PBS but depending on the protein other buffers such as Hepes, HBS or TRIS buffer can also be used to prepare the protein solution.
  b. The protein solution can be diluted or concentrated slightly ranging from 20 to 200 µg/ mL.

- ✓ Do not dilute Pro-DeliverIN ™ reagent. If small quantities are required, prepare a higher amount of Antibody/Pro-DeliverIN™ and dispense the appropriate volume in your dish.

- ✓ Medium or buffer without serum & supplement must be used for the mixture preparation. Culture medium such as DMEM or OptiMEM are recommended. In contrast, we do not recommend RPMI.

- ✓ Pro-DeliverIN ™ reagent can be used onto cells in absence of serum. In this case, replace the complete culture medium by serum-free medium. This procedure can be more efficient to deliver certain proteins in some cells. After 3-4h, add some serum-containing medium if further incubation time is necessary.

- ✓ For suspension cells, gently mix complexes to the cell solution by pipeting the medium up and down (3-4 times) to ensure a uniform distribution of the mixture. It is important to promote the contact of the complexes with cells during this mixing procedure. In addition, this favours the disruption of potential clumps of cells that are preventing the complexes to get access to all cells

IMPORTANT NOTE

The presence of BSA as additives in antibody reagent (present in a lot of commercially available antibodies) can completely inhibit the antibody delivery. If BSA is present in your antibody sample, we recommend removing it before proceeding with the delivery assay (see details in the complete instruction manual). Sodium azide has only insignificant effect with the indicated amounts of antibodies used. The presence of glycerol in antibody solution does not interfere with the antibody delivery experiment.

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

www.ozbiosciences.com

Any questions?

tech@ozbiosciences.com

Package content	PI10100: 100 µL of Pro-DeliverIN PI10250: 250 µL of Pro-DeliverIN PI10500: 500 µL of Pro-DeliverIN PI11000: 1mL of Pro-DeliverIN **NOTE:** Pro-DeliverIN is provided with 100µL of R-Phycoerythrin Positive Control
Shipping conditions	Room Temperature
Storage conditions	Store the Pro-DeliverIN transfection reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product description	Pro-DeliverIN is a lipopolyamine formulation specifically designed to achieve intracellular delivery of biologically active proteins.
Important notice	For research use only. Not for use in diagnostic procedures

1. Cells preparation

Adherent cells. 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 80-90% confluent (percentage of growth surface covered with cells) at the time of experiment (refer to Table 1).

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
6 well	2.5 – 5 x 10^5	5 – 20 x 10^5	1.8 mL

Table 1: Recommended number of cells to seed

Suspension cells. For fast growing cells, split the cells the day before the protein delivery experiment at a density of 2 to 5 x 10^5 cells / mL, so they are maintained in excellent condition.

2. Protein/Pro-DeliverIN complexes preparation

a. *Protein*: Prepare a protein solution at 100µg/mL in PBS.

b. *Pro-DeliverIN*: Add 0.8 to 10µL of Pro-DeliverIN in an empty microtube (Refer to Table 2).

Tissue Culture Dish	Protein Quantity (µg)	Dilution Volume (µL)	Pro-DeliverIN Volume (µL)	Transfection Volume
96 well	0.4	20	0.8	200 µL
24 well	1	100	2	500 µL
6 well	5	200	10	2 mL

Table 2: Recommended protein amount, Pro-DeliverIN volume and transfection conditions

c. Add the recommended protein quantity (see Table 2) to the Pro-DeliverIN and mix by pipetting up & down

d. Incubate at Room Temperature for 10 to 15 min.

3. Transfection

a. Add 20 to 200µL of serum-free medium to the antibody / Pro-DeliverIN™ mixture (refer to Table 2) and disperse immediately drop by drop the complexes onto cells growing in their regular culture medium. Gently rock the plate to ensure a uniform distribution.

b. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of protein delivery efficiency.

IMPORTANT NOTE

R-Phycoerythrin is provided in the Pro-DeliverIN ™ kit as a positive control. Use 2 µL of Pro-DeliverIN ™ per 1 µg of protein for the delivery assay. This control protein is provided to help you setting up your experiment for your particular cell type. Because proteins are very different one from another, reflecting a variety of physical properties, optimum conditions determined to deliver the control protein may differ from the conditions that should be used to deliver your protein of interest.

Optimization Protocol

In order to get the best out of the *Pro-DeliverIN* ™ reagent, several parameters can be optimized:

- Volume of Pro-DeliverIN ™ reagent - This depends on the protein, the presence or not of contaminants or additives, and on the cell type.
- Protein amount and concentration - which depends on the protein itself and on the sensitivity of the assay.
- Presence or absence of serum during the delivery experiment - For all the proteins tested we did not observed an important influence of this parameter. However, the background is reduced when serum is present during the delivery experiment
- Cell type and density - Best results are reached when cells are 50–70 % confluent at the delivery time.
- Incubation time - As assays are type dependent we recommend performing a time-course experiment to set up the optimal incubation time which will vary with protein activity, half-life...

We recommend that you optimize the different parameters starting from the conditions given in the protocol above within the range indicated in the Table 3.

Tissue Culture Dish	Protein Quantity (µg)	Pro-DeliverIN ™ (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
6 well	2.5 - 10	2.5 - 25	200	2 mL

Table 3: Optimization of protein amount and volume of Pro-DeliverIN ™ reagent

1) Start by optimizing the volume of the Pro-DeliverIN ™ reagent with your protein and particular cell type (refer to Table 3). To this end, use a fixed amount of protein and vary the amount of the Pro-DeliverIN ™ reagent. For instance, from 0.5 to 5 µL of Pro-DeliverIN ™ reagent in a 24-well plate with 1 µg of protein.

2) Thereafter, increase the amount of protein to be delivered maintaining constant the ratio Pro-DeliverIN / protein determined above. Note that in some cases, you get better results by increasing the amount of protein while maintaining constant the volume of the Pro-DeliverIN ™ reagent.

3) After having identified the optimal quantities of Pro-DeliverIN ™ and protein, you can pursue the process by optimizing other parameters such as the cell number (density), the time course of your experiment…

Additional products

- **Ab-DeliverIN** for Antibody Delivery
- **Pro-DeliverIN CRISPR** optimized for recombinant Cas9 protein delivery

Purchaser Notification

Limited License
The purchase of the Pro-DeliverIN kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of protein as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the Pro-DeliverIN kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all Pro-DeliverIN kit reagents and documentation to OZ Biosciences, or by destroying all Pro-DeliverIN components. Purchasers are advised to contact OZ Biosciences with the notification that a Pro-DeliverIN kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the Pro-DeliverIN kit research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations
Pro-DeliverIN kit and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

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