

To find the ideal conditions, Pro-DeliverIN™ must be tested at ratios of **1 $\mu\text{L}/\mu\text{g}$** , **2 $\mu\text{L}/\mu\text{g}$** , and **2.5 $\mu\text{L}/\mu\text{g}$** (μL of Pro-DeliverIN / μg of Protein). For the protein quantity, we suggest **0.4 μg** per well in 96-well, **1 μg** per well in 24-well and **5 μg** per well in 6-well.

1. Prepare 3 wells: seed the cells to be at 70% confluent on the day of transfection.



2. Prepare the protein solution at 100 $\mu\text{g}/\text{mL}$ in PBS

The presence of BSA as additives in protein reagent can completely inhibit the protein delivery. In case of BSA, remove it before proceeding.



3. Prepare 3 tubes of Pro-DeliverIN



96-well plate
0.4 μL /0.8 μL /1 μL

24-well plate
1 μL /2 μL /2.5 μL

6-well plate
5 μL /10 μL /12.5 μL

4. Add the protein solution to each tube of Pro-DeliverIN



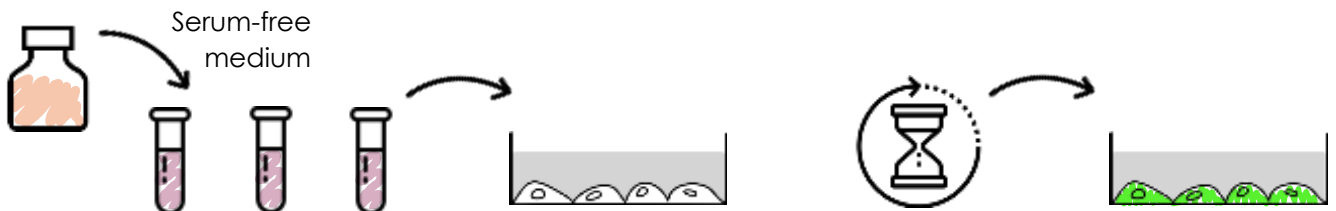
Incubate 10 min at RT

96-well plate
0.4 μg (4 μL of 100 $\mu\text{g}/\text{mL}$ per well)

24-well plate
1 μg (10 μL of 100 $\mu\text{g}/\text{mL}$ per well)

6-well plate
5 μg (50 μL of 100 $\mu\text{g}/\text{mL}$ per well)

5. Add serum-free medium to each tube and distribute to cells



Up to 20 μL (96-well), 100 μL (24-well) or 200 μL (6-well).

Distribute each mix dropwise onto cells

Incubate 3 to 48h at 37°C

Choose the best ratio Protein:Pro-DeliverIN

Contact us regarding optimization procedures or for any technical question at: tech@ozbiosciences.com or visit our website: www.ozbiosciences.com.

Before you begin

Pro-DeliverIN™ is a lipopolyamine formulation specifically designed to achieve intracellular delivery of biologically active proteins.

Protocol | General considerations

- Allow reagents to reach room temperature and gently vortex them before forming complexes.
- Depending on protein properties, the amount tested can be doubled.
- R-Phycoerythrin is provided as a positive control: use 1 or 2 µL Pro-DeliverIN per µg of protein for delivery assay.

⚠ Do not dilute Pro-DeliverIN before use.

⚠ We recommend preparing the complexes in a 0.5 mL tube to keep working volumes low.

⚠ Prepare protein dilution at 100 µg/mL in PBS. Do not use culture medium for this step. Protein concentration may vary depending on the application.

1. Cells preparation:

One day before transfection prepare the cells according to table 1 (recommended 70% confluence).

Cell culture format	Adherent cell nb. / well	Suspension cell nb. / well
96-well	0.05-0.15 x 1.10 ⁵	0.5-1.0 x 1.10 ⁵
24-well	0.5-1 x 1.10 ⁵	1.5-5 x 1.10 ⁵
6-well	2-5 x 1.10 ⁵	5-20 x 1.10 ⁵

Table 1: Cell number suggested per well.

2. Preparation of Protein/Pro-DeliverIN complexes:

a. *Protein:* Prepare protein solution at 100 µg/mL in PBS and add the desired quantity into an empty tube.

b. *Pro-DeliverIN:* Add 0.8 to 10 µL of Pro-DeliverIN to the protein solution (refer to Table 2).

c. Gently mix by pipetting up & down and incubate the mixture at room temperature for **10 to 15 min.**

⚠ Do not vortex or centrifuge.

3. Transfection:

a. Add 20 to 200 µL serum-free medium to the protein/Pro-DeliverIN mix (refer to table 2).

b. Disperse immediately drop-by-drop the complexes onto cells in their regular culture medium.

d. Gently rock the plate and cultivate the cells at **37°C** in a CO₂ incubator under standard conditions until evaluation of delivery efficiency (3 to 48h).

Cell culture format	Protein quantity	Pro-DeliverIN volume	Dilution volume	Transfection volume
96-well	0.4 µg	0.4 µL	20 µL	200 µL
24-well	1 µg	2 µL	100 µL	500 µL
6-well	5 µg	10 µL	200 µL	2 mL

Table 2: Recommended Protein amounts, Pro-DeliverIN volumes and transfection conditions. For other formats, adjust conditions accordingly.

Protocol Optimization

Contact us regarding protocols for optimization procedures, or any technical question:

✉ - tech@ozbiosciences.com.

Use, handling and storage

For Research Use Only. Not for use in humans. Not for use in diagnostic or therapeutic purposes.

Shipping conditions: Room Temperature

Storage conditions Pro-DeliverIN: +4°C

Shelf life: 1 year from the date of purchase

Kit contents

PI10100 : 100 µL of Pro-DeliverIN reagent

PI10250 : 250 µL of Pro-DeliverIN reagent

PI10500 : 500 µL of Pro-DeliverIN reagent

PI11000 : 1 mL of Pro-DeliverIN reagent

Pro-DeliverIN is provided with 100 µL of R-Phycoerythrin positive control.

Certificate of analysis on demand.

Related Products

Ref	Description
#PIC60500	Pro-DeliverIN CRISPR
#PHY0100	R-Phycoerythrin (100 µg/mL) in DPBS pH 7.4
#AI11000	Ab-DeliverIN antibody delivery reagent

Purchaser Notification | Conditions of Sale

This product is sold in accordance with our general conditions of sale that you can find on our website: <https://ozbiosciences.com/content/3-terms-and-conditions>.