Pro-DeliverIN™ CRISPR
Protein Delivery Inside Living Cells

To find the ideal conditions, Pro-DeliverIN™ CRISPR must be tested at ratios of **1 µL/µg**, **2 µL/µg**, and **2.5 µL/µg** (µL of Pro-DeliverIN CRISPR / 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 µg/mL in PBS – Cas9 endonuclease or Cas9/gRNA RNP complexes

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 CRISPR

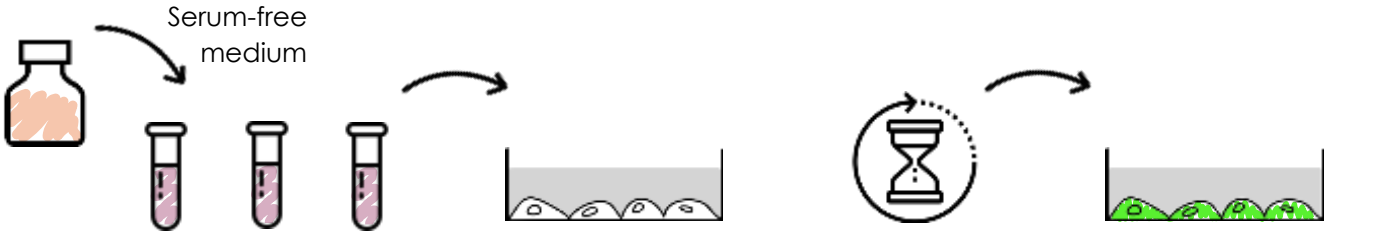
96-well plate	24-well plate	6-well plate
0.4 µL/0.8 µL/1 µL	1 µL/2 µL/2.5 µL	5 µL/10 µL/12.5 µL

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

Incubate 10 min at RT

96-well plate	24-well plate	6-well plate
0.4 µg (4 µL of 100 µg/mL per well)	1 µg (10 µL of 100 µg/mL per well)	5 µg (50 µL of 100 µg/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 CRISPR

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

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Pro-DeliverIN™ CRISPR
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Before you begin

Pro-DeliverIN™ CRISPR 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 CRISPR before use.
⚠ We recommend preparing the complexes in a 0.5 mL tube to keep working volumes low.
⚠ Prepare protein/ RNP 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 or RNP complexes solution at 100 µg/mL in PBS and add desired quantity into an empty tube.

b. Pro-DeliverIN CRISPR: Add 0.8 to 10 µL of Pro-DeliverIN CRISPR 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 CRISPR 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 CO_2 incubator under standard conditions until evaluation of delivery efficiency (3 to 48h).

Cell culture format	Protein or RNP quantity	Pro-DeliverIN CRISPR vol.	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 CRISPR 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 CRISPR: +4°C
Shelf life: 1 year from the date of purchase

Kit contents

PIC60100 : 100 µL of Pro-DeliverIN CRISPR reagent
PIC60500 : 500 µL of Pro-DeliverIN CRISPR reagent
CAS9PIC : 50 µg Cas9 nuclease + 100 µL Pro-DeliverIN CRISPR reagent

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

Certificate of analysis on demand.

Related Products

Ref	Description
#PI11000	Pro-DeliverIN protein delivery reagent
#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.

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