

# Pro-DeliverIN CRISPR transfection reagent



## Protocol

*For CRISPR/ CAS9 - Genome Editing using Cas9 protein  
or Cas9/gRNA RNP complexe.*

## ProDeliverIN CRISPR Reagent User Guide

Package contents	<b>PIC60100:</b> 100µl of ProDeliverIN CRISPR reagent <b>PIC60500:</b> 500µl of ProDeliverIN CRISPR reagent Pro-DeliverIN™ CRISPR is provided with 100µL of R-Phycoerythrin Positive Control.
Storage Conditions	Store at 4°C upon receipt
Product Description	ProDeliverIN CRISPR kit is a transfection reagent optimized for recombinant Cas9 protein delivery or Cas9/gRNA RNP complexes. For your gene editing applications, this reagent provides high transfection efficiency with minimal toxicity.
Important notice	For Research use only. Not for use in diagnostic procedures

### BEFORE YOU BEGIN:

- Protein or Protein/RNP complex solution and Pro-DeliverIN CRISPR should be used at room temperature and gently vortexed prior to use.
- All the complexes must be prepared in medium without serum and supplement.
- It is not recommended to use RPMI during complex preparation, prefer DMEM or PBS.
- For sensitive cells, medium can be replaced with fresh complete culture medium 4 to 6h after transfection.

Tissue Culture Dish	Cell Number per well	Protein or Protein/RNP complex quantity per well*	Total transfection volume per well
96-well	0.5 – 2.0 × 10 <sup>4</sup>	0.4 µg	0.2 mL
24-well	0.5 – 1.0 × 10 <sup>5</sup>	1 µg	0.5 mL
12-well	1.0 – 2.0 × 10 <sup>5</sup>	2 µg	1.0 mL
6-well	2.0 – 4.0 × 10 <sup>5</sup>	5 µg	2.0 mL
60 mm dish	0.5 – 1.0 × 10 <sup>6</sup>	10 µg	4.0 mL
90-100 mm dish	1.0 – 2.0 × 10 <sup>6</sup>	30 µg	8.0 mL
T75 flask	2.0 – 5.0 × 10 <sup>6</sup>	35 µg	12.0 mL

Table 1: Recommended cell number, protein or protein/RNP complex and transfection volume per well.

## PROTOCOL STEPS

The following protocol is given for a single well of a 24-well tissue culture plate containing  $\sim 1 \times 10^5$  cells/well in 400  $\mu\text{L}$  complete culture serum. If a different culture plate format is used, adjust cell number and reagent amounts according to the table 1.

### NOTES:

-> Pro-DeliverIN CRISPR (PI) should be stored at  $+4^\circ\text{C}$ . Use 2  $\mu\text{L}$  of PI per  $\mu\text{g}$  of Cas9 endonuclease or Cas9/gRNA RNP complex.

-> To find the ideal conditions, Pro-DeliverIN CRISPR must be tested at ratios 1  $\mu\text{L}/\mu\text{g}$  protein, 2  $\mu\text{L}/\mu\text{g}$  protein and 2.5  $\mu\text{L}/\mu\text{g}$  protein.

-> For the protein quantity, we suggest 0.4  $\mu\text{g}$  per well in 96-well, 1  $\mu\text{g}$  per well in 24-well and 5  $\mu\text{g}$  per 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  $\mu\text{g}$  of protein instead of 1  $\mu\text{g}$  for 2 / 4 / 5  $\mu\text{L}$  of Pro-DeliverIN CRISPR).

### 1. Cas9 protein solution

Prepare a suspension of Cas9 endonuclease or RNA complexes at 100  $\mu\text{g}/\text{mL}$  in PBS.

### 2. Pro-DeliverIN CRISPR solution

Add 2  $\mu\text{L}$  of Pro-DeliverIN CRISPR in a new tube.

### 3. Complex preparation

Mix 10  $\mu\text{L}$  of protein or protein/RNP complex solution (1  $\mu\text{g}$ ) with Pro-DeliverIN CRISPR solution. Mix gently by carefully pipetting up and down.

Incubate the mixture for 20 min at room temperature.

### 4. Transfection

Add the complexes dropwise onto the cells and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture. Incubate the cells under your standard culture conditions for 3 to 48 h.

### OPTIONAL:

Perform a medium change 2 to 6h after transfection. Withdraw the transfection medium and add fresh growth medium

## Additional products for CRISPR Cas9 experiments:

- RmesFect CRISPR for mRNA transfection
- PolyMag CRISPR for Genome editing using expression plasmids
- ViroMag CRISPR to enhance transduction efficiency of CRISPR/Cas9 viruses

### Purchaser Notification

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