Pro-DeliverIN™

INSTRUCTION MANUAL

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Pro-DeliverIN ™ - Protein Delivery Reagent

Instruction Manual

Pro-DeliverIN ™ - Protein Delivery Reagent has been designed to transport a variety of proteins inside living cells.

List of **Pro-DeliverIN ™** Kits

Catalog Number	Description	Volume (µL)	Number of experiments / 24 well-plates	Number of experiments / 6 well-plates
PI10100	Pro-DeliverIN™	100	50-100	10-20
PI10250	Pro -DeliverIN™	250	125-250	25-50
PI10500	Pro -DeliverIN™	500	250-500	50-100
PI11000	Pro -DeliverIN™	1000	500-1000	100-200

Each kit contains 10 µg of R-Phycoerythrin at a concentration of 100 µg / mL in PBS.

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1. Technology

1.1. Description

Congratulations on your purchase of the **Pro-DeliverIN ™ - Protein Delivery Reagent**!
The delivery of proteins inside living cells represents an alternative to nucleic acids transfection and a powerful strategy for functional studies or therapeutic approaches. This new and innovative reagent opens new fields of investigation in rising field of proteomics to elucidate complex molecular mechanisms or to design new potential therapy. For example, the intracellular delivery of apoptose-related protein can assist in elucidating programmed cell death mechanism as well as cancer resistance to drug treatment. The proteins delivered inside cells with **Pro-DeliverIN ™** retain their structure and function, there is no need to covalent linking, just mix the protein delivery reagent with your protein of interest. **Pro-DeliverIN ™** is a lipid-based formulation which forms non-covalent complexes with proteins. Complexes are internalized by cells and proteins are released into the cytoplasm without any cytotoxicity.

Principal **Pro-DeliverIN ™** advantages:

1. Efficient protein delivery in a wide variety of cells including primary cells
2. Versatile: various proteins were delivered inside cells
3. Ready to use reagent
4. High cell viability - No cytotoxicity (biodegradable lipids)
5. Rapid and straightforward procedure
6. Compatible with and without serum-containing media

1.2. Kit Contents

Kit contents vary according to their size:

- 1 tube containing 0.1 mL of Pro-DeliverIN ™ Reagent good for 50-100 assays in a 24-well plate.
- 1 tube containing 0.25 mL of Pro -DeliverIN ™ Reagent good for 125-250 assays in a 24-well plate.
- 1 tubes containing 0.5 mL of Pro -DeliverIN ™ Reagent good for 250-500 assays in a 24-well plate.
- 1 tubes containing 1 mL of Pro -DeliverIN ™ Reagent good for 500-1000 assays in a 24-well plate.
- Each kit contains 10 µg of R-Phycoerythrin at a concentration of 100 µg / mL in PBS.

Stability and Storage. Upon receipt and for long-term use, store all reagent tubes at +4°C.
Pro-DeliverIN ™reagent and R-Phyco positive control - Protein Delivery Reagent kits are stable for at least 1 year at the recommended storage temperature.
Shipping condition Room Temperature

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2. Applications

2.1. Protein Delivery

Delivery systems allowing exogenous proteins to be transported inside living cells represent a major interest. It opens novel strategies to assess functions of proteins or to elucidate new molecular mechanisms. Some approaches based on the use of PTD (Peptide Transduction Domain) were developed successfully to transduce proteins across the plasma membrane. However, these PTD poorly interact with proteins and covalent linkage between the protein and PTD is required. **Pro-DeliverIN ™** is a formulation of lipids able to capture proteins through electrostatic and hydrophobic interactions and deliver them inside cells. Several recombinant proteins were efficiently delivered in a wide variety of cells with the **Pro-DeliverIN ™ - Protein Delivery Reagent.** The proteins assayed were B and R-Phycoerythrin, BSA, β-galactosidase, human active caspase-3 and various immunoglobulins labeled or not with different *fluorophores*: FITC, TRITC, AlexaFluor®488 and AlexaFluor®546.

Important criteria for efficient protein delivery inside cells.

It is obvious that proteins differ one from another in term of size, structure, composition, property and activity. Contrary to nucleic acids which have all the exact same bio-physical properties, association of proteins with the Pro-DeliverIN ™ reagent is variable. Thus, optimal delivery conditions for one particular protein cannot be translated to another type of protein. In the same way, some proteins might not be efficiently delivered with the Pro-DeliverIN ™reagent, due to their specific properties. For instance, some proteins are very difficult to deliver inside cells such as very basic proteins having an elevated isoelectric point. However, there are definitely no pre-establish rule to determine whether a specific protein can be delivered or not. Thus, we highly encourage you to try and evaluate this reagent with your protein of interest. Moreover, delivery efficiency can vary from one cell to another. Consequently, do not hesitate to contact us, we will be delighted to provide advices or comments about the potential Pro-DeliverIN ™ reagent efficiency with your protein of interest.

2.2. Cell Types and Targets

Pro-DeliverIN ™ - Protein Delivery Reagent is applicable with numerous cell types and multiple proteins. This reagent has been successfully tested on a variety of immortalized cell lines as well as some primary cells.

Cell Line	***Cell Type***	***Source***
3T6	Embryonic fibroblasts	Mouse
A549	Non-small cell lung carcinoma	Human
B16-F10	Melanoma	Mouse
BEAS-2B	Bronchial epithelial cells	Human
BHK21	Fibroblasts (Kidney)	Hamster
CHO-K1	Epithelial-like (Ovary)	Hamster
COS-1, COS-7	Fibroblasts (Kidney)	Green Monkey
HaCaT	Keratinocytes	Human
HEK-293	Transformed Embryonic (Kidney)	Human
HeLa	Cervical Epithelial Carcinoma	Human
Jurkat	T cell leukemia (lymphoma)	Human
L929	Fibrosarcoma	Mouse
K562	Myelogenous leukemia	Human
MDCK	Epithelial (Kidney)	Canine
N2A	Neuroblastoma	Mouse
NIH3T3	Fibroblasts	Mouse
Raw264.7	Monocytes/macrophages	Mouse
U87	Glioblastoma	Human
Vero 10A1	Epithelial (Kidney)	Monkey
Primary cells		
Neurons		Rat
Glial cells		Rat

3. Protocol

3.1. General Considerations

The instructions given below represent sample protocols that were applied successfully on a variety of cells. Our R&D team has extensively tested and optimized the **Pro-DeliverIN ™ reagent** 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 or not of additives and contaminants has a high impact on the delivery efficiency. Consequently, we advise you to optimize the delivery parameters in order to achieve the best effects. Several optimization protocols are provided in the Appendix.

Important Parameter: 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 or other similar additives does not interfere with the protein delivery experiment. Preservative such as sodium azide could lead to some cytotoxicity if present in high concentration. However, we have never observed unwanted effects due to the presence of sodium azide with the indicated amounts of proteins used. Generally, the final concentration of sodium azide added onto cells is very low and negligible. Otherwise, it can be removed by dialysis.

3.2. Cell 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 (see the suggested cell number in the table 1).

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.

Table 1: Recommended number of cells to seed.

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
12 well	1 – 2 x 10^5	2.5 - 10 x 10^5	900 µL
6 well	2.5 – 5 x 10^5	5 – 20 x 10^5	1.8 mL
60 mm dish	5 – 10 x 10^5	1 – 5 x 10^6	3.8 mL
90 - 100 mm	12 – 30 x 10^5	2.5 – 10 x 10^6	7.6 mL
T-75 flask	15 – 40 x 10^5	5 – 15 x 10^6	9.6 mL

3.3. 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 depending on the protein used other buffer such as Hepes, HBS or Tris can also be used.
- Note. The presence of a small amount of glycerol (1-5% in the 100 µg / mL solution), currently used for protein storage, does not interfere with protein delivery into cells. 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.
- The protein solution can be diluted or concentrated slightly ranging from 20 to 200 µg / mL.

2) Add 0.4 to 70 µL of **Pro-DeliverIN ™** reagent in one microtube, according to the table 2.

- Be careful to add the reagent in the bottom of the microtube without touching the wall of the tube which will result in reagent loss.
- Do not dilute **Pro-DeliverIN** ™ reagent. Accordingly, if pipeting of small quantities is required (especially for 96-well plate), we recommend preparing higher amount of protein - **Pro-DeliverIN** ™ complexes and thereafter dispense the appropriate volume (amount of protein) in your well or dish.
- The table 2 presented below was used to deliver various proteins in different cell lines. It can be used as a starting point. ***However, some optimization may be needed (see table 3 in appendix for optimization range).***

3) Add 4 to 350 µL of protein (100 µg / mL) to the **Pro-DeliverIN** ™ reagent, according to the table 2, and mix by pipeting up and down several times.

4) Incubate 10-15 min at room temperature.

Table 2: Suggested amount of protein and **Pro-DeliverIN** ™ reagent.

Tissue Culture Dish	Protein Quantity (µg)	Pro-DeliverIN ™ (µL)	Dilution Volume (µL)	Total Medium Volume
96 well	0.4	0.8	20	120 µL
24 well	1	2	100	500 µL
12 well	2	4	100	1 mL
6 well	5	10	200	2 mL
60 mm dish	10	20	200	4 mL
90 - 100 mm	30	60	400	8 mL
T-75 flask	35	70	400	10 mL

5) Add 20 to 400 µL (see dilution volume in table 2) of serum-free medium to the protein / **Pro-DeliverIN** ™ mixture and disperse immediately onto the cells growing in their regular culture medium (with serum).

- **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. Hepes, HBS or TRIS buffer can be used instead of PBS to prepare the protein solution if you use this procedure. 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 favors the disruption of potential clumps of cells that are preventing the complexes to get access to all cells.

6) Incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of the protein delivery efficiency (3-48h). Incubation time will depend on different parameters (assay, half life of the protein delivered...)

<u>Important note</u>: 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.

4. Appendix

4.1. 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.
- Dilution buffer of the protein. PBS is recommended but other buffers (TRIS, Hepes, HBS...) can be more appropriate depending on proteins.
- Presence or absence of serum during the delivery experiment. For all the proteins tested we did not observe an important influence of this parameter.
- 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.

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

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
12 well	1 - 4	1 - 10	100	1 mL
6 well	2.5 - 10	2.5 - 25	200	2 mL
60 mm dish	5 - 20	5 - 50	200	4 mL
90 - 100 mm	15 - 60	15 - 120	400	8 mL
T-75 flask	20 - 80	20 - 160	400	10 mL

1) Start by optimizing the volume of the **Pro-DeliverIN** ™ reagent with your protein and particular cell type (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...

4.2. Example of protocol: β-galactosidase delivery

1) Seed 75,000 A549 cells / well in a 24-well plate the day before the protein delivery experiment.

2) Dilute the β-galactosidase protein in PBS at 100 µg / mL (β-galactosidase used was from Calbiochem).

3) Add 1.5 µL of the **Pro-DeliverIN** ™ reagent in one microtube.

4) Add 10 µL of -galactosidase (100 µg / mL) diluted solution into **Pro-DeliverIN** ™ vial.

5) Mix by pipeting up and down 3-4 times.

6) Incubate 10 min at room temperature.

7) Add 100 µL of serum-free medium to the protein / **Pro-DeliverIN** ™ mixture and disperse immediately onto the cells growing in their regular growth culture medium (with serum).

8) Incubate the cells at 37°C in a CO_2 incubator under standard conditions during 4-6h.

9) Fix the cells with 2 % paraformaldehyde.

10) Analyze the delivery efficiency by staining the cells with X-Gal (*Catalog number GX-10003, OZ Biosciences*).

4.3. Quality Controls

To assure the performance of each lot of **Pro-DeliverIN ™ - Protein Delivery Reagent** produced, we qualify each component using rigorous standards. The following assays are conducted *in vitro* to qualify the function, quality and activity of each kit component.

Specification	Standard Quality Controls
Purity	Silica Gel TLC assays. Every compound shall have a single spot.
Sterility	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 7 days.
Biological Activity	Delivery of R-Phycoerythrin in NIH3T3 cells monitored by cytofluorimetry and fluorescence microscopy. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot.

4.4. Troubleshooting

Problems	Comments and Suggestions
Low delivery efficiency	1- **Protein purity**. Make sure that the recombinant protein is highly pure and devoid of additives such as BSA or detergents. 2- **Pro-DeliverIN ™ amount.** Optimize the quantity of Pro-DeliverIN ™ reagent as described in the table 3. 3- **Pro-DeliverIN ™ / protein ratio**. Optimize the Pro-DeliverIN ™ / protein ratio within the range indicated in table 3. 4- **Protein amount**. Use different amount of protein with the recommended or optimized (above) Pro-DeliverIN ™ / protein ratio. 5- **Cell density.** A non-optimal cell density at the time of protein delivery can lead to insufficient uptake. The optimal confluence should range from 50 to 70%. 6- **Cell condition.** 1) Cells that have been in culture for a long time (> 8 weeks) may become resistant to the delivery. Use freshly thawed cells that have been passaged at least once. 2) Cells should be healthy and assay during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) alters considerably the delivery efficiency. 7- **Cell culture medium composition.** For some cells, protein delivery efficiency can be increased without serum or under reduced serum condition. Thus, assay these cells in serum-free medium during the first 4h of incubation. 8- **Medium used for preparing Pro-DeliverIN ™ / protein complexes**. Change the protein dilution buffer and/or the pH to improve the delivery. Highly basic proteins are difficult to deliver due to the presence of positive charges but this can be compensated by the protein hydrophobic property. The charge of the protein can be modified with the pH. Only use serum free medium to prepare the complexes. 9- **Incubation time and transfection volume.** 1) The optimal time range between delivery and assay varies with cells, type of protein, kinetics of biological function, etc. The delivery efficiency can be monitored after 4 to 96h. R-Phycoerythrin can be used to quantitatively monitored delivery kinetics. 2) To increase delivery efficiency, transfection volume suggested can be reduced for the first 4 to 24 hours.

	10- **Old Pro-DeliverIN ™ / protein complexes.** The Pro-DeliverIN ™ reagent / protein complexes must be freshly prepared every time. Complexes prepared and stored for more than 1 hour can be aggregated. Depending on the protein, reduce this time to avoid the aggregation which may occur during the complex formation. 11- **Positive control.** Ensure that your experiment is properly set up and includes a positive control. The R-Phycoerythrin provided in the kit can be used as positive control for delivery efficiency. 12- **Pro-DeliverIN ™ reagent temperature.** Reagents should have an ambient temperature and be vortexed prior to use. 13- **Pro-DeliverIN ™ reagent storage.** Delivery efficiency can slowly decrease if Pro-DeliverIN ™ reagent is kept more than one week at room temperature.
Cellular toxicity Cellular toxicity	1- **Concentration of Pro-DeliverIN ™ / protein too high.** Decrease the amount of Pro-DeliverIN ™ / protein complexes added to the cells by lowering the protein amount or the Pro-DeliverIN ™ reagent. Complexes aggregation can cause some toxicity; prepare them freshly and adjust the ratio as outlined previously. 2- **Unhealthy cells.** 1) Check cells for contamination, 2) Use new batch of cells, 3) Ensure culture medium condition (pH, type of medium used, contamination etc), 4) Cells are too confluent or cell density is too low, 5) Verify equipments and materials 3- **Protein is cytotoxic.** Use suitable controls such as cells alone, Pro-DeliverIN ™ reagent alone or mock delivery (with positive R-Phycoerythrin provided). 4- **Incubation time.** Reduce the incubation time of complexes with the cells. Delivery medium can be replaced by fresh medium after 3 to 24 h if necessary. 5- **Protein quality**. Use high quality protein as impurities could lead to cell death. 6- **Key protein delivered.** If the protein delivered impacts cell survival this can lead to cell death, for instance as demonstrated with the recombinant caspase-3. In this way, the cell death is induced by the proteases.

5. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)* Mega Magnetic plate *(mega size to hold 4 culture dishes at one time)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine™ kit: Lipofectamine™ 2000 + CombiMag (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag *(for siRNA application)*
Transfection enhancer:
CombiMag *(to improve any transfection reagent efficiency)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(specific for Retrovirus and Lentivirus)*
AdenoMag *(for Adenoviruses)*
In vivo Magnetofection
In vivo ViroMag (for magnetic assisted viral infection)
In vivo PolyMag (polymer-based magnetic nanoparticles)
In vivo DogtorMag (lipid-based magnetic nanoparticles)
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
VeroFect *(for Vero cells)*
Ecotransfect *(Economical reagent for routine transfection)*
FlyFectin *(for Insect cells)*
i-MICST TECHNOLOGY
Viro-MICST (*to transduce directly on magnetic cell purification columns)*
3D TRANSFECTION TECHNOLOGY
3DfectIN *(for hydrogels culture)*
3Dfect *(for scaffolds culture)*
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (*for **H**igh **Y**ield **P**rotein **E**xpression)*
PLASMIDS PVECTOZ
pVectOZ-LacZ / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase
ASSAY KITS
Bradford – Protein Assay Kit MTT cell proliferation kit β-Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, K^+ and Na^+ 1g G-418, Sulfate 1g
X-Gal powder 1g

Purchaser Notification

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We Bring The World Of Biotechnology To You

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www.bocascientific.com
(781) 686-1631
info@bocascientific.com