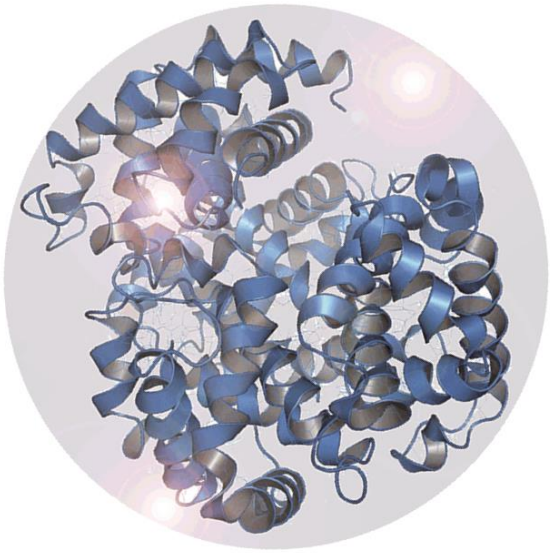


Protein Delivery

Ab-DeliverIN™

Antibody Delivery Inside Living Cells

Protocol

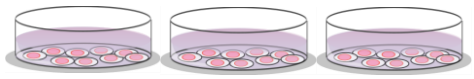
OZBIOSCIENCES
The art of delivery systems

Ab-DeliverIN™ Quick Protocol

To find the ideal conditions, Ab-DeliverIN™ must be tested at ratios **1 µL/µg**, **2 µL/µg** and **2.5 µL/µg** of Ab-DeliverIN™ / Antibody. For the antibody 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*.

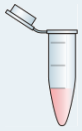
1

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



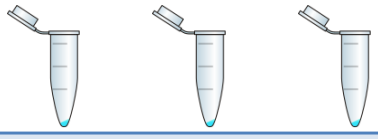
2

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



3

Prepare 3 tubes of Ab-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

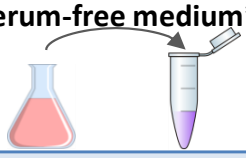
Add the antibody solution (step 2) to each tube of Ab-DeliverIN™ (step 3)

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

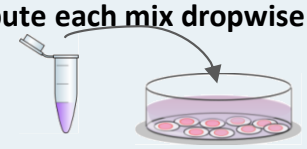
Add serum-free medium* to each tube



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

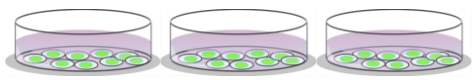
6

Distribute each mix dropwise onto the cells



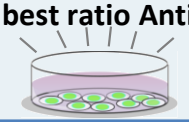
7

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



8

Choose the best ratio Antibody:Ab-DeliverIN™



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

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ Depending on the properties of your antibody, the amount used in the test can be doubled (*i.e* in a 24 well plate, 2 µg of antibody instead of 1 µg for 2 / 4 / 5 µL of Ab-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 antibody solution. Dilute the antibody to be delivered in PBS at 100 µg / mL.
  - *Do not use culture media for this step!* Prefer use PBS to prepare antibody solution than other buffers such as Hepes, HBS or TRIS buffer
  - The antibody solution can be diluted or concentrated slightly ranging from 50 to 200 µg/ mL.

- ✓ Do not dilute Ab-DeliverIN™. If small quantities are required, prepare a higher amount of Antibody/Ab-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.

- ✓ Ab-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

Ab-DeliverIN Reagent | Specifications

Package content	AI20100: 100 µL of Ab-DeliverIN AI20250: 250 µL of Ab-DeliverIN AI20500: 500 µL of Ab-DeliverIN AI21000: 1mL of Ab-DeliverIN Ab-DeliverIN is provided with 100µL of FITC-labeled IgG Positive Control
Shipping conditions	Room Temperature
Storage conditions	Store the Ab-DeliverIN transfection reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product description	Ab-DeliverIN is a lipopolyamine formulation specifically designed to achieve intracellular delivery of biologically active Antibodies.
Important notice	For research use only. Not for use in diagnostic Procedures

1. Cell Preparation

Adherent cells. It is recommended to seed or plate the cells the day prior the antibody 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 antibody delivery experiment at a density of 2 to 5 x 10^5 cells / mL, so they are maintained in excellent condition.

2. Antibody/Ab-DeliverIN complexes preparation

a. *Antibody*: Prepare an antibody solution at 100µg/mL in PBS.

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

Culture vessel	Antibody Quantity (µg)	Final dilution Volume (µL)	Ab-DeliverIN Volume (µL)	Transfection Volume
96 well	0.4	20	0.4	200 µL
24 well	1	100	2	500 µL
6 well	5	200	10	2 mL

Table 2: Suggested antibody amount, Ab-DeliverIN volume and transfection conditions

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

d. Incubate at room temperature for 10 to 15 minutes.

3. Transfection

a. Add 20 to 200µL of serum-free medium to the antibody / Ab-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 antibody delivery efficiency (3 to 48h).

IMPORTANT NOTE

FITC-labeled IgG is provided in the Ab-DeliverIN™ kit as a positive control. Use 1 or 2 µL of Ab-DeliverIN™ per 1 µg of antibody for the delivery assay. This control antibody is provided to help you to set up your experiment for your particular cell type.

Optimization Protocol

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

- Volume of Ab-DeliverIN ™ reagent. This depends on the antibody, the presence or not of contaminants or additives, and on the cell type.
- Presence or absence of serum during the delivery experiment. For all the antibodies 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 cell density. Best results are achieved when cells are 50–70 % confluent at the time of the delivery.
- Incubation time. Assays type dependent. Perform a time-course experiment to set up the optimal incubation time since binding of antibody to its target is dependent on the target localization and accessibility as well as the protein turnover rate.

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

Tissue Culture Dish	Antibody Quantity (µg)	Ab-DeliverIN ™ (µL)	Final dilution Volume (µL)	Total Medium Volume
96 well	0.2 - 0.5	0.2 - 1	20	120 µL
24 well	0.5 - 3	0.5 - 5	100	500 µL
6 well	2.5 - 15	2.5 - 25	200	2 mL

Table 3: Optimization of antibody amount and volume of Ab-DeliverIN ™ reagent

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

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

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

NOTES

Additional Products for your protein delivery experiments

- **Pro-DeliverIN** for protein delivery
- **Pro-DeliverIN CRISPR** for Cas9 protein delivery

Purchaser Notification

Limited License
The purchase of the Ab-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 nucleic acids 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 Ab-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 Ab-DeliverIN kit reagents and documentation to OZ Biosciences, or by destroying all Ab-DeliverIN components. Purchasers are advised to contact OZ Biosciences with the notification that a Ab-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 Ab-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
Ab-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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