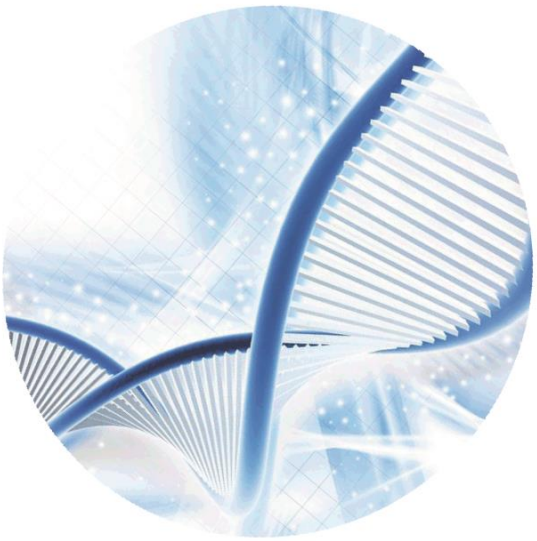

Transfection reagent

Helix-IN™

DNA Delivery

CHAMP Technology (Cationic Hydroxylated Amphiphilic Multi-block Polymer)

Protocol

We Bring The World Of Biotechnology To You

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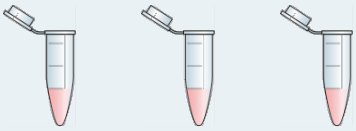
OZBIOSCIENCES
The art of delivery systems

Helix-IN™ Quick Protocol

To find the ideal conditions, **Helix-IN™** must be tested at ratio **1.5:1** (**1.5 µL/µg** DNA). We suggest **3** different DNA amounts depending on cell type (refer to table 1).

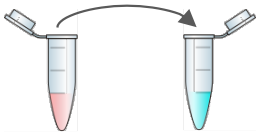
1 **Seed cells to be at 70% confluent the day of transfection***

2 **Prepare 3 tubes of DNA according to table 1**



96 well plate	24 well plate	6 well plate
DNA in 15µL of serum-free medium or buffer*	DNA in 50µL of serum-free medium or buffer*	DNA in 200µL of serum-free medium or buffer*

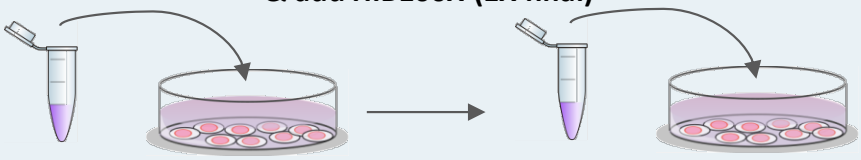
3 **Add Helix-IN™ directly into the DNA solution (volume depends on DNA quantity)**



96 well plate	24 well plate	6 well plate
Helix-IN ratio 1.5:1 in 15 µL of DNA	Helix-IN ratio 1.5:1 in 50 µL of DNA	Helix-IN ratio 1.5:1 in 200 µL of DNA

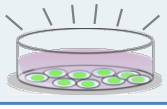
4 Incubate **30 min** at room temperature

5 **Distribute each mix dropwise onto the cells to insure uniform distribution & add HIB100X (1X final)**



6 **Incubate cells for 24 to 72h at 37°C until evaluation of transgene expression**

7 **Choose the best ratio DNA: Helix-IN™**



These conditions might require some further optimizations depending on your cells, DNA, RNA, etc.

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ For cell lines, seed the cells 24h before transfection in a 96-well plate, 24-well plate or 6-well plate in respectively 150 µL, 400 µL and 2 mL of complete culture medium.

- ✓ **DNA quantity**
  You may adjust the amount of DNA depending on the transfected cell lines.
  Due to the high performance of Helix-IN reagent, we recommend using low doses of DNA for "easy-to-transfect" cell lines such as HEK-293, COS, CHO or HeLa cells. For more challenging cell lines (NIH-3T3, C2C12 for example), you may consider to increase the amount of DNA (refer to Table 1 below).

- ✓ **Helix-IN is very versatile:** with low amounts of DNA use high volumes of reagent and use lower ratios of Helix-IN with high DNA doses.

		96-well plate (15 µL)		**24-well plate** (50 µL)		**6-well plate** (200 µL)	
		DNA (µg)	Hx (µL)	**DNA (µg)**	Hx (µL)	**DNA (µg)**	Hx (µL)
Easy-to-transfect HEK-293, CHO, COS, HeLa…	Low DNA amount	**0.03**	0.045	**0.125**	0.18	**0.5**	0.75
		0.06	0.09	**0.25**	0.37	**1**	1.5
		0.125	0.18	**0.5**	0.75	**2**	3
Hard-to-transfect C2C12, MCF7, MDA-MB-231…	High DNA amount	**0.125**	0.18	**0.5,**	0.75	**2**	3
		0.18	0.27	**0.75**	1.12	**3**	4.5
		0.25	0.375	**1.0 µg**	1.5	**4**	6

Table 1: Suggested DNA amount (per well) and Helix-In volumes depending on the cell line and the plate format

- ✓ **Transfection reagent/DNA ratios**
  Ratios of 1:1 to 2.5:1 work with most of the cell types; however other ratios from 3:1 to 6:1 can be used for depending on cell types or applications.

- ✓ Allow reagents to reach RT and gently vortex them before forming complexes.

- ✓ Do not incubate complexes Helix-IN/DNA less than 30 min at RT.

- ✓ **Medium without serum & supplement** must be used for the DNA/Helix-IN complexes preparation. Culture mediums such as DMEM (with or w/o phenol red), RPMI (with or w/o phenol red), DMEM-F12, alpha-MEM, EMEM or OptiMEM are recommended.

- ✓ **Avoid using buffers** (HBS, PBS, NaCl, …) or H2O for preparing the complexes.

- ✓ Dilute the reagent with deionized water for doses of Helix-IN™ less than 1µL.

- ✓ For some cells, 24h post-transfection, replace the medium with fresh pre-warm medium or just add fresh growth culture medium to the cells. In the case of cells very sensitive to transfection, the medium can be replaced after 3-4h.

Package content	HX10100: 100µL of Helix-IN + 1mL HIB Enhancer reagent HX10500: 500µL of Helix-IN + 5mL HIB Enhancer reagent HX11000: 1mL of Helix-IN + 10mL HIB Enhancer reagent
Shipping conditions	Room Temperature
Storage conditions	Store Helix-IN transfection reagent and HIB100X at -20°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product Descriptions	Helix-IN is a polymeric formulation specifically designed to achieve high transfection efficiency in a broad spectrum of cell lines, difficult-to-transfect and primary cells
Important notice	For research use only. Not for use in diagnostic procedures

1. Cells preparation

It is recommended to plate the cells the day prior transfection* in classical culture medium. Cells should be 60-80 % confluent at the time of transfection (refer to Table 1). The correct choice of optimal plating density also depends on the planned time between transfection and protein expression analysis: for a large interval, we recommend a lower density and for a short interval a higher density may be advantageous.

Tissue Culture Dish format	Surface area per well[1]	Cell Number
96 wells	0.32 cm^2	0.2 – 0.4 x 1.10^5
24 wells	2 cm^2	0.5 – 0.8 x 1.10^5
6 wells	10 cm^2	2 – 4 x 1.10^5

[1] Surfaces area may vary depending on the manufacturer

Table 2: Suggested cell number (per well)

NOTE: Some primary cells require being prepared 48H before transfection; change half of the culture medium 24h transfection

2. DNA/Helix-IN complexes preparation

a. *DNA*: Dilute the indicated quantity of DNA (refer to Table 3) in 15 to 200 µL of culture medium without serum and supplement. Prepare two tubes to tests two DNA amounts.

Tissue Culture Dish	DNA Quantity (µg)	Helix-IN ratio (µL)	Dilution Volume (µL)	HIB100X 1 X final (µL)	Transfection Volume
96 well	0.06 & 0.2	1.5:1	15	2.0	200 µL
24 well	0.25 & 0.5	1.5:1	50	5.0	500 µL
6 well	1 & 3	1.5:1	200	20.0	2 mL

Table 3: Suggested DNA amount, Helix-IN volumes and transfection conditions

b. *Helix-IN*: Vortex the reagent and add indicated quantities of Helix-IN (refer to Table 3) directly into 15 to 200 µL of the corresponding DNA solution. See optimization protocol for other Helix-IN/DNA ratios.

c. Mix gently by carefully pipetting up and down. Do not vortex.

d. Incubate at room temperature for 30 min.

3. Transfection

a. Add the complexes onto cells drop by drop and gently rock the plate to ensure a uniform distribution.

b. Add HIB100X at 1X final directly onto the cells.

c. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of transgene expression.

IMPORTANT OBSERVATION TO ENSURE MAXIMUM OF EFFICIENCY

DNA amounts and Helix-IN volumes are given as a starting point for most of transfection conditions. Specific cell lines or application may need to optimize nucleic acid quantities and ratio. Refer to optimization protocol at the end of this protocol.

IMPORTANT OBSERVATION FOR PROTEIN PRODUCTION OVER 24H

In case of protein production experiment over 24h, we recommend using two times more amounts of DNA per well to yield maximal levels of protein.

Protocol | DNA Co-transfection

For co-transfection of several plasmids DNA, mix different amounts of each plasmid to reach the recommended total DNA quantity and transfect as described above. For example, if you have two DNA plasmids and need a stoichiometry of 2:1, mix 0.25 µg of each plasmid, complex the 0.5 µg of DNA with 1 µL of Helix-IN.

Option for co-transfection

Transfections can be realized sequentially instead of simultaneously. So, cells can be transfected with one plasmid DNA first and 4h to 24h later can be transfected with the other plasmid DNA. Follow the procedure as detailed above for DNA transfection. A medium changed can be also performed between the two transfections.

Optimization Protocol

1. General considerations

To achieve the highest efficiency, optimize the transfection conditions as follows:

- **Vary the Helix-IN (µL) / DNA (µg) ratio from 1:1 to 2.5:1.**
  We recommend trying 1.0, 1.5, 2.0, and 2.5 µL Helix-IN per µg DNA.

NOTE: other ratios from 3:1 to 6:1 can be used for specific cell types or applications.

- Once the optimal ratio Helix-IN/DNA ratio is found, adjust the DNA quantity according to Table 4.
- Use high ratios of reagents with low DNA doses and low ratios of Helix-IN with high DNA amounts
- Finally, culture medium composition (for preparing the complexes), cell density, total culture medium volume and incubation times can also be optimized.

Tissue Culture Dish	DNA Quantity (µg)
96 well	0.05 to 0.25
24 well	0.25 to 2.0
6 well	1 to 4

Table 4: Suggested range of DNA amounts for optimization (per well)

2. Optimization protocol for 24 well plates

Depending on the lab, culture medium, cell clone… conditions may be optimized to achieve the best transfection.

KEY PARAMETERS BEFORE BEGINNING

- For low volumes, to ensure a correct pipetting, we recommend preparing a 5X dilution of Helix-IN in sterile culture-grade H_2O
- Discard remaining dilution after use.

a. Find the ideal ratio of Helix-IN

Use fixed amount of DNA and vary volume of Helix-IN.

- Prepare a 0.5 µg/well DNA solution enough for 5 wells: dilute 2.5 µg DNA in 250 µL medium w/o supplement.
- Prepare 4 tubes containing 50 µL of the DNA solution
- Add 2.5 and 3.8 µL, of 5X diluted Helix-IN to two DNA tubes and 1 and 1.25 µL to the other two tubes (corresponding to ratios of 1:1, 1.5:1, 2:1 and 2.5:1, respectively)
- Incubate 30 min at RT
- Add the complexes onto the cells in a drop wise manner and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.
- Add 5 µL of HIB100X per well and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture
- Incubate the cells under standard culture conditions for 24 to 72 h.

b. Find the ideal amount of DNA

Once the ratio is found, keep it unchanged and optimize conditions to find ideal DNA amount.

- Prepare 4 DNA solutions containing 0.3, 0.5, 0.75 and 1 µg DNA in 50 µL medium w/o supplement*.
- For each solution add Helix-IN corresponding to the ratio found in (a).
- Incubate 30 min at RT
- Add the complexes onto the cells in a drop wise manner and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture
- Add 5 µL of HIB100X per well and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture
- Incubate the cells under standard culture conditions for 24 to 72 h.

*For easy-to-transfect cell lines, we recommend using 1.5 times less DNA.