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

1. Prepare 3 wells: seed the cells to be at 70% confluent on the day of transfection



2. Prepare 3 tubes of DNA <u>according to table 1</u>



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

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

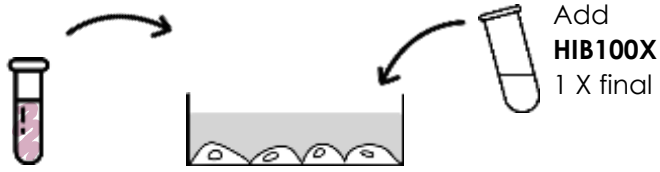


96-well plate	24-well plate	6-well plate
Helix-IN (1.5:1) in 15 µL DNA	Helix-IN (1.5:1) in 50 µL DNA	Helix-IN (1.5:1) in 200 µL DNA

4. Incubate 30 min at room temperature and distribute onto cells & add HIB100X

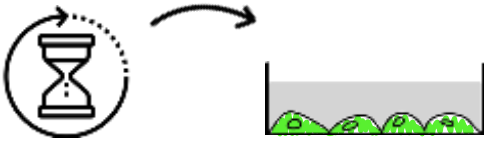


Incubate 30 min at RT



Distribute each preparation of complexes in each well.

5. Incubate the cells for 24 to 72 h at 37°C until evaluation of the transgene expression



Incubate **24** to **72 h** at 37°C

Choose the best ratio DNA:Helix-IN

Contact us regarding protocols for DNA transfection in suspension cells, optimization procedures or for gene silencing with siRNA at: **tech@ozbiosciences.com** or visit our website: **www.ozbiosciences.com**.

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Helix-IN™

CHAMP Technology (Cationic Hydroxylated Amphiphilic Multi-Block Polymer)

Before you begin

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.

Protocol | General considerations

• Allow reagent to reach room temperature (RT).
• For doses of Helix-IN less than 1µL, dilute the reagent with deionized water only.
• Adjust DNA amount depending on the target cells: low doses are recommended for easy-to-transfect cells and high doses for more challenging ones (refer to Table 1).
⚠ Classic ratios of 1:1 to 2.5:1 work with most cell types; higher ratio (3:1 to 6:1) can be used depending on the cell type or the applications.
⚠ Do not incubate complexes less than 30 min at RT.

	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, COS7...)	**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...)	**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**	1.5	**4**	6

Table 1: Suggested DNA amounts per well and Helix-IN depending on the cell line and the plate format. Easy- and Hard-to-transfect cells require respectively low or high DNA amounts.

1. Cells preparation:
One day before transfection prepare the cells according to table 2 (60-80% confluence).

Cell culture format	Surface area per well	Adherent cell nb. / well
96-well	0.32 cm^2	0.2-0.4 x 1.10^5
24-well	2 cm^2	0.5-0.8 x 1.10^5
6-well	10 cm^2	2-4 x 1.10^5

Table 2: Suggested cell number per well.

2. Preparation of DNA/Helix-IN complexes:
a. DNA: Prepare three tubes to test three DNA amounts: dilute the indicated quantity of DNA in **15** to **200 µL** of culture medium w/o supplement (Table 3).

b. Helix-IN: Vortex the reagent and add Helix-IN (ratio **1.5:1**) directly into the DNA solutions (Table 3).

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

d. Incubate at Room Temperature for **30 min**.

3. Transfection:
a. Add the Helix-IN/DNA complexes onto cells drop by drop and gently rock the plate to ensure a uniform distribution.

b. Add HIB100X (1X final) to the cells.

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

Cell culture format	DNA quantity (µg)	Helix-IN ratio	Dilution volume	HIB100X (1X final)	Transfection volume
96-well	0.03 to 0. 25	1.5:1	15 µL	2 µL	200 µL
24-well	0.125 to 1.0		50 µL	5 µL	500 µL
6-well	0.5 to 4		200 µL	20 µL	2 mL

Table 3: Recommended DNA amounts, Helix-IN volumes and transfection conditions. For other formats, adjust conditions accordingly.

Protocol | Optimization | Co-transfection

Contact us regarding protocols for co-transfection, optimization procedure, 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 Helix-IN & **HIB100X:** -20°C

Shelf life: 1 year from the date of purchase

Kit contents

HX10100 : 100 µL of Helix-IN reagent + 1 mL HIB100X
HX10500 : 500 µL of Helix-IN reagent + 5 mL HIB100X
HX11000 : 1 mL of Helix-IN reagent + 10 mL HIB100X

Certificate of analysis on demand.

Related Products

Ref	Description
#DG80500	DreamFect Gold transfection reagent
#PL00120	pVectOZ-GFP control plasmid

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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