Helix-IN™ Transfection Kit

INSTRUCTION MANUAL

DNA Transfection

OZBIOSCIENCES
The art of delivery systems

Helix-IN™

Instruction Manual

Helix-IN™ is the most powerful broad-spectrum transfection reagent from classic cell lines to difficult-to-transfect cells.

List of Helix-IN™ kits

Catalog Number	Description	Helix-IN Volume (µL)	HIB100X Volume	Number of transfections[1]
HX10100	Helix-IN™ transfection kit	100	1 mL	50-100
HX10500	Helix-IN™ transfection kit	500	5 mL	250-500
HX11000	Helix-IN™ transfection kit	1 mL	2x5 mL	500-1000

[1] Values are given for transfections in a 24-well plate using the recommended transfection conditions (0.5 µg DNA/well)

Use the content of the table above to determine the appropriate catalog number for your needs. You can order these products by contacting us (order@ozbiosciences.com). For all other supplementary information, do not hesitate to contact our dedicated technical support (tech@ozbiosciences.com).

OZ Biosciences SAS
163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09 - France
Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515
contact@ozbiosciences.com
order@ozbiosciences.com

OZ Biosciences INC
4901 Morena Blvd
Suite 501
San Diego CA 92117 - USA
Ph : + 1-858-246-7840
Fax : + 1-855-631-0626
contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com

www.ozbiosciences.com

1. Technology

1.1. Description

Helix-IN™ is the most powerful transfection reagent developed by OZ Biosciences. **Helix-IN™** is centered on the latest polymer-based transfection technologies that allow preserving membrane stability for higher viability while improving transfection efficiency.

Helix-IN™ transfection kit principal advantages:

- Broad spectrum transfection reagent for cell lines & difficult-to-transfect cells
- Need of low nucleic acid amount
- Minimized toxicity due to a new class of polymer based reagent
- High level of nucleic acid compaction
- Increased protein production
- Easy and straightforward protocol
- Compatible with any culture medium.

1.2. Kit Contents, Stability and Storage

Contents

Kits content varies according to their size:

- 1 tube containing 100 µL of Helix-IN™ and 1 mL of HIB100X good for up to 50-100 assays in a 24-well plate.
- 1 tube containing 500 µL of Helix-IN™ and 5 mL of HIB100X good for up to 250-500 assays in a 24-well plate
- 1 tube containing 1 mL of Helix-IN™ and 2x5 mL of HIB100X good for up to 500-1000 assays in a 24-well plate

Each kit contains one vial of Helix-IN™ reagent and one vial of HIB100X enhancer reagent.

Stability, Storage and Shipping

Stability: Helix-IN™ and HIB100X are stable for at least 12 months at the recommended storage temperatures.
Storage: Upon reception and for long-term use, store the Helix-IN™ transfection reagent and HIB100X at -20°C.
Shipping condition: Room Temperature

Helix-IN™ transfection reagent is stable for several days at room temperature or at +4°C without losing activity. The number of freeze and thaw cycles does not affect the efficiency of the reagent.

2. Applications

Helix-IN™ has been developed for **DNA transfection into a broad spectrum of cells**; from classic cell lines to difficult-to-transfect cells. This polymer-based transfection reagent is serum compatible and can be used for transient as well as stable transfection. Helix-IN™ and HIB100X are very stable, ready-to-use and intended for research purpose only.

3. General Protocol

3.1. General Considerations / Important Guidelines

The instructions given below represent standard protocol. Optimal conditions may vary depending on the plasmid, cell lineage, clone, size of cell culture dishes and conditions of culture.
As a starting point, use **1 to 2.5 µL of Helix-IN™ per µg of DNA.** Addition of **HIB100X** at **1X final**, although recommended, is still optional. Refer to the optimization procedure to find optimal transfection conditions.

- **Cells** should be healthy and assayed during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) will considerably affect the transfection efficiency. Use regularly passaged cell lines or freshly prepared primary cells at confluence between 60 and 80% (visual confluence). Do not use cells that have been cultured for too long (> 2 months).
- **Nucleic Acids** should be as pure as possible and free of contaminants. We suggest avoiding long storage of the diluted nucleic acid solution before the addition of Helix-IN™ to circumvent any degradation or surface adsorption. We recommend using pVectOZ-GFP plasmid as an efficient transfection control.

- **Culture Medium**. The exclusion of antibiotics from the media during transfection has been reported to enhance gene expression levels. We did not observe a significant effect of the presence or absence of antibiotics with the Helix-IN™ transfection reagent.
- **Transfection conditions:** it is often not necessary to increase the amount of DNA to increase transfection efficiency. 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 depending on cell types or applications.
- **IMPORTANT NOTES:**

  (1) 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 lowering the DNA amount 1.5 times 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 suggested in the quick protocol up to 2 times.

Table 1: Suggested DNA amount (per well) and Helix-IN volumes (ratio 1.5:1) depending on cell type and the plate format

		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

(2) 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.
(3) Allow reagents to reach RT and gently vortex them before forming complexes.
(4) Do not incubate complexes Helix-IN/DNA less than 30 min at RT.
(5) 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.
(6) Avoid using buffers (HBS, PBS, NaCl, ...) or H2O for preparing the complexes.

3.2. Cells Preparation

Cell culture prior to transfection: one day before transfection prepare the cells according to the Table 2. 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 (see the suggested cell number in the Table 2). 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.

Table 2: Cell number suggested (per well).

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

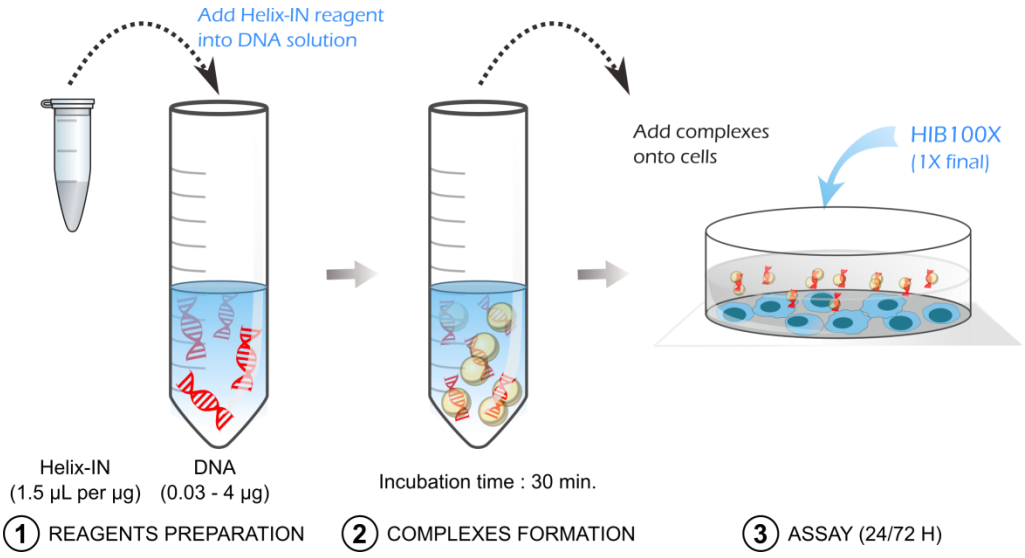
[1] Surfaces area may vary depending on the manufacturer.

* Some primary cells require being prepared 48H before transfection; change half of the culture medium 24H transfection.

3.3. DNA Transfection Protocol

Follow the standard procedure to transfect DNA. The Table 1 shows optimized transfection conditions according to different cell culture formats and cell type (all amounts are given on per-well basis).
Use this rapid protocol: **1.5 µL of Helix-IN™ per µg of DNA (ratio 1.5:1).**

NOTE: We suggest beginning with the recommended ratios and optimize it, if required.



1) **Reagents preparation**

Allow reagents to reach room temperature before beginning.

a. *DNA solution.* Dilute DNA in **15** to **200 µL** of culture medium without any supplement (SVF, antibiotics, growth factors...). Refer to table 3.

b. *Helix-IN™ solution.* Mix the reagent gently before use and add the required volumes directly into DNA solution.

2) **Complexes formation**

Incubate the mixture for **30 minutes** at room temperature. Do not vortex or centrifuge!

NOTE: Proceed to step 3 within 30 minutes

3) **Transfection**

a. Add the complexes in a dropwise manner onto the cells growing in complete culture medium and homogenize by rocking the plate back and forth to ensure a uniform distribution of the mixture.

b. Add **2.0** to **20.0 µL** of HIB100X (1X final) directly onto the cells.

4) **Assay**

Incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of the protein expression. We recommend performing assay from 24 to 72h.

Table 3: DNA amount, Helix-IN™ volume and transfection conditions suggested (per well).

Tissue Culture Dish format	DNA Quantity (µg) [1]	Helix-IN™ Volume (µL)[2]	Dilution Volume (µL)[3]	HIB100X (µL)	Total culture medium Volume
96 wells	0.03 – 0.25	1.5 µL per µg 2.0 µL per µg	15	2.0	200 µL
24 wells	0.125 – 1.0		50	5.0	500 µL
6 wells	0.5 – 4.0		200	20.0	2 mL

[1] **DNA quantity depends on cell type, refer to table 1 for DNA amount to use**

[2] For low volumes, to ensure a correct pipetting, we recommend preparing dilution of Helix-IN™ in sterile culture-grade H2O.

[3] Volumes of dilution medium for step 1.

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.

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.

Reverse transfection. Prepare the complexes as described above, then transfer them into an empty culture dish or well and finally and directly add the cells at twice the recommended cell density.

Other protocols for optimization or co-transfection are also available on our website at www.ozbiosciences.com or by contacting our technical support department (tech@ozbiosciences.com).

OZ Biosciences offers plasmids coding for CAT (#PL00010), GFP (#PL00020), LacZ (#PL00030), LUC (#PL00040) and SEAP (#PL00050) as transfection controls. These control plasmids are recommended to set up optimization procedure.

3.4. Optimization protocol for DNA transfection

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 Helix-IN/DNA ratio is found, adjust the DNA quantity according to Table 4.
- Finally, culture medium composition (for preparing the complexes), cell density, total culture medium volume and incubation times can also be optimized.

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

Tissue Culture Dish format	DNA Quantity (µg)
96 well	**0.03 to 0.25**
24 well	**0.125 to 2**
6 well	**0.5 to 4**

Refer to detailed optimization protocols for 24-well plates the end of this document (section 7).

4. Appendix

Our dedicated and specialized technical support team will be pleased to answer any of your requests at tech@ozbiosciences.com. In addition, do not hesitate to visit our website www.ozbiosciences.com.

4.1 Quality Controls

To assure the performance of each lot of Helix-IN™ reagent produced, we qualify each component using rigorous standards. The following *in vitro* assays are conducted to qualify the function, quality and activity of each component.

Specification	Standard Quality Controls
Sterility	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 15 days.
Biological Activity	Transfection efficacies on COS7 & NIH-3T3 cells. Every lot shall have an acceptance specification of > 85% of the activity of the reference lot.

4.2. Troubleshooting

Problems	Comments and Suggestions
Low transfection efficiency	1- **Optimization of Helix-IN/ DNA ratio.** See section 3.4. 2- **DNA amount.** Use different quantities of DNA with the optimized ratio. 3- **Cell density.** A non-optimal cell density at the time of transfection can lead to insufficient uptake. The optimal confluency should range from 60 to 80% but most favorable cell density may vary according to the cell subtype; preferably mid-log growth phase. 4- **DNA quality.** Nucleic acid should be as pure as possible. Free of contaminants (proteins, phenol, ethanol etc.) and endotoxins. 5- **Type of promoter**. Ensure that DNA promoter can be recognized by the cells to be transfected. Use pVectOZ plasmids as controls for transfection. 6- **Cell condition.** 1) Cells in culture for a long time (> 8 weeks) may become resistant to transfection. Use freshly thawed cells that have been passaged at least once. 2) The presence of contaminants (mycoplasma, fungi) alters considerably the transfection efficiency. 7- **Medium used for preparing DNA / transfection reagent complexes**. It is critical to use serum-free medium or buffer (HBS, PBS) during the complexes preparation. 8- **Culture medium composition.** 1) In some cases, transfection efficiency can be increased in absence of serum. Transfect these cells in serum-free medium during the first 4h. 2) The presence of antibiotics might affect cell health and transfection efficiency. 9- **Incubation time and transfection volume.** 1) The optimal time range between transfection and assay varies with cells, promoter, expression product, etc. The transfection efficiency can be monitored after 18h depending on the readout and the cell. 2) To increase transfection efficiency, transfection volume suggested can be reduced for the first 24 hours. 10- **Old transfection reagent / DNA complexes.** The transfection reagent / DNA complexes must be freshly prepared each time to avoid aggregation. 11- **Transfection reagent temperature.** Reagents should be at ambient temperature and be vortexed prior to use.

Cellular toxicity	1- **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. 2- **Protein expression is toxic.** Use suitable controls such as cells alone, transfection reagent alone or mock transfection with a control plasmid. 3- **DNA quality - Presence of contaminants.** Ensure that nucleic acid is pure, contaminant-free and endotoxin-free. Use high quality nucleic acids as impurities can lead to cell death. 4- **Concentration of transfection reagent / nucleic acid too high.** Decrease the amount of nucleic acid / reagent complexes added to the cells by lowering the nucleic acid amount or the transfection reagent concentration. Complexes aggregation can cause some toxicity; prepare them freshly and adjust the ratio as outlined previously.

5. Related Products

MAGNETOFECTION TECHNOLOGY

Super Magnetic Plate *(standard size for all cell culture support)*
+ **Transfection reagents**:
PolyMag Neo - *for all nucleic acids*
NeuroMag - *dedicated for neurons*
SilenceMag - *for siRNA applications*

PLASMIDS PVECTOZ

pVectOZ-LacZ, Luc, CAT, GFP, SEAP

ASSAY KITS

FluoProdige Protein Assay Kit – Fluorometric assay for protein & peptide quantification
MTT cell proliferation kit
ROS Detection Assay Kit – Quantify cellular Reactive Oxygen Species (ROS)
β-Galactosidase assay kits (CPRG/ONPG)

BIOCHEMICALS

D-Luciferin, K^+ and Na^+
X-Gal powder 1g / G-418, Sulfat

Our dedicated and specialized technical support group will be pleased to answer any of your request and to assist you in your experiments. Do not hesitate to contact us for all complementary information and remember to visit our website in order to stay inform on our last breakthrough technologies and updated on our complete product list.

6. Purchaser Notification

Limited License

The purchase of the Helix-IN™ transfection kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in section 1, Kit Contents). Reagents are intended **for in-house research only** by the buyer. Such use is limited to 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 Helix-IN™ transfection kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact the Director of Business Development at OZ Biosciences.

Buyers may end this License at any time by returning all Helix-IN™ transfection kit material and documentation to OZ Biosciences, or by destroying all Helix-IN™ kit components. Purchasers are advised to contact OZ Biosciences with the notification that a Helix-IN™ transfection 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 Helix-IN™ transfection 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

The Helix-IN™ transfection 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.

For more information, or for any comments on the terms and conditions of this License, please contact:

Director of Business Development
OZ Biosciences SAS
Parc Scientifique et Technologique de Luminy
163, avenue de Luminy, zone entreprise, case 922
13288 Marseille Cedex 9, France
Tel: +33 (0)4.86.94.85.16
Fax: +33 (0)4. 86.94.85.15
E-mail: business@ozbiosciences.com

7. Optimization Procedure (4-well plate)

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.

1) 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.

2) 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 **(1)**.
- 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.