

**HYPE-5™ Transfection Kit**  
**INSTRUCTION MANUAL**

**HYPE-5™**  
**Transfection Kit**

***High Yield Protein Expression***  
***BioProduction***



**OZ BIOSCIENCES**  
The art of delivery systems

# HYPE-5™ Transfection Kit

## Instruction manual

**HYPE-5™** Transfection Kit achieves **High Yield Protein Expression** in mammalian cells.

It has been designed for maximum protein production in HEK293 and CHO suspension cells growing in chemically defined synthetic media.

### List of HYPE-5™ Kits

Catalog Number	HYPE-5 reagent	HYPE-Blast
HY01500	1 x 1.5 mL	1 x 5 mL
HY03000	2 x 1.5 mL	2 x 5 mL
HY15000	1 x 15 mL	1 x 50 mL
HY30000	2 x 15 mL	2 x 50 mL

1.5 mL of HYPE-5 reagent is suitable for 0.5 to 1 Liter of cell culture.

Please inquire about a custom quote for bulk quantities.

For CHO cells, HYPE-5 and HYPE-Blast are required to achieve highest protein production yield whereas for HEK293 cells HYPE-Blast reagent is optional.

Both reagents (HYPE-5 and HYPE-Blast) are also available individually:

Catalog Number	HYPE-5 reagent
HYR10003	2 x 1.5 mL
HYR10015	1 x 15 mL
HYR20030	2 x 15 mL

Catalog Number	HYPE-Blast
HYB00005	1 x 5 mL

Use the content of the tables above to determine the appropriate catalog number for your needs. You can order these products by contacting us (telephone, fax, mail, e-mail) or directly through our website. For all other supplementary information, do not hesitate to contact our dedicated technical support ([tech@ozbiosciences.com](mailto:tech@ozbiosciences.com)).

### OZ BIOSCIENCES

Parc Scientifique de Luminy; 163 avenue de Luminy,  
Zone entreprise, case 922  
13288 Marseille Cedex 9, France  
Tel: +33 (0) 486 948 516  
Fax: +33 (0) 486 948 515

E-mail: [contact@ozbiosciences.com](mailto:contact@ozbiosciences.com) Web Site: [www.ozbiosciences.com](http://www.ozbiosciences.com)



# 1. Introduction

## 1.1. Description

Congratulations on your purchase of the **HYPE-5™** Transfection Kit!

**HYPE-5™** Transfection Kit is the newest reagent dedicated to achieve **High Yield Protein Expression** in mammalian cells. This kit has been designed for maximum efficiency in HEK293 and CHO cells growing in suspension and cultivated in chemically defined medium. These efficient transient expression systems represent an ideal and quicker alternative to the stable expression systems (costly and time-consuming). Scale-up to larger volumes for production of milligrams of protein per liter of cell culture is straightforward and easy with simple and cost efficient handling steps.

**HYPE-5™** Kit presents unique properties:

1. High protein production yield.
2. Suitable for both HEK293 and CHO suspension cells.
3. Compatible with any synthetic or regular media.
4. Ideal for bioreactor, spinner or flasks.
5. Rapid, simple to scale up and ready-to-use.
6. Free from animal sources.

HYPE-5™ Kit has been designed for maximum efficiency in HEK293 and CHO cells growing in suspension (flask, spinner and bioreactor) but it is also suitable for numerous cells. Please do not hesitate to contact us at [tech@ozbiosciences.com](mailto:tech@ozbiosciences.com) for any further information or enquiry.

## 1.2. Kit Contents

OZ Biosciences offers four sizes of HYPE-5™ Transfection Kit:

Catalog Number	HYPE-5 reagent	HYPE-Blast	Total volume of cell culture
HY01500	1 x 1.5 mL	1 x 5 mL	0.5-1L
HY03000	2 x 1.5 mL	2 x 5 mL	1-2L
HY15000	1 x 15 mL	1 x 50 mL	5-10L
HY30000	2 x 15 mL	2 x 50 mL	10-20L

For CHO cells, both HYPE-5 reagent and HYPE-Blast are required to achieve highest protein production yield whereas for HEK293 cells HYPE-Blast is optional. Both reagents (HYPE-5 and HYPE-Blast) are available individually (see catalog number above). These reagents are purely synthetic and do not contain any components of animal origin.

### Stability and Storage

**Storage:** Upon reception and for long-term use, store HYPE-5 reagent at -20°C and HYPE-Blast at +4°C. Both reagents are stable for at least one year under recommended storage conditions.

**Shipping condition:** Room Temperature

## 1.3. Cells and Culture Medium

HYPE-5™ Kit has been used and validated with cells from different origins (FreeStyle™ CHO-S/293-F cells from invitrogen, CHO protein free from ECACC...). It is suitable for any kind of mammalian cells used to produce proteins. This Kit has been tested with several chemically defined media (FreeStyle™ CHO-S/293 Expression medium, ProCHO 4 medium from Lonza or EX-CELL™ ACF CHO Medium from Sigma-Aldrich...). It is compatible with any specific media for protein production.

Do not use culture medium containing high antibiotic level (up to 0.5 X penicillin/streptomycin final concentration) or high Pluronic® surfactant concentration (up to 0.01% w/v final concentration) because it could have dramatic impact on protein production level.

## 1.4. Important Considerations

The instructions given below represent protocols that were applied successfully with HEK293 and CHO cells growing in suspension and cultivated in chemically defined medium. Optimal conditions may vary depending on the nucleic acid, cell types, growth condition (medium, size of cell culture...). Therefore we suggest optimizing the various parameters as described in section 4.

However, to obtain good data rapidly, you can start by following our rapid protocol as guidelines. The use of HYPE-Blast is optional. We observed, when using HYPE-Blast, a large increase in protein expression with CHO suspension cell model and no influence with HEK293 suspension cell. So, we suggest testing, during the optimization procedure, whether or not the use of HYPE-Blast increases the protein production. If this step is not performed we always recommend using the HYPE-Blast as describe in the rapid protocol.

Before starting you will need the following materials:

- Protein production cell model growing in suspension
- Chemically defined or regular medium for cell culture
- Serum free medium for complexes preparation
- Highly purified endotoxin-free plasmid DNA
- HYPE-5 reagent and HYPE-Blast
- Polycarbonate and sterile Erlenmeyer flask with vented cap
- Orbital shaker in 37°C incubator with a humidified atmosphere of 8% CO<sub>2</sub>.

## 2. Rapid Protocol

**The following protocol is suitable for a 30 mL cell culture volume.** Transient transfection experiments may be performed in a larger volume, allowing large-scale protein production. For other culture size refers to the Table 1 in the next section. For 30mL volume, we recommend the 125 mL flask.

Key parameters before beginning the procedure:

- The DNA, HYPE-5 and HYPE-Blast solutions should have an ambient temperature and be gently vortexed prior to use.
- Do not use serum-containing medium for preparing the complexes!
- Cell culture maintenance: we suggest sub-culturing the cells at a density of 0.05-0.2 x 10<sup>6</sup> cells/mL for each passage (48-72 h). Avoid high cell density and keep cell growth conditions consistent for reproducibility.
- Cells must be actively dividing and in exponential growth, mainly as single cells, before transfection for maximum efficiency.

### 1) Cell preparation

18-24 hours before transfection, dilute the cells to 0.6-0.8 x 10<sup>6</sup> cells/mL and incubate on orbital shaker (~125 rpm) at 37°C, 8% CO<sub>2</sub>. The day of transfection, dilute the cells to 1 x 10<sup>6</sup> cells/mL (cell density should be about 1.2-1.5 x 10<sup>6</sup> cells/mL). Transfer 30 mL of cell culture in every 125 mL Erlenmeyer flask.

### 2) Complexes preparation

- 2.1. DNA solution. Dilute 45 µg of DNA in 600 µL of serum free medium. Incubate 5 min at room temperature.
- 2.2. HYPE-5 solution. Dilute 90 µL of HYPE-5 reagent in 600 µL of serum free medium. Incubate 5 min at room temperature.
- 2.3. Add the DNA solution into the HYPE-5 solution, mix gently by carefully pipetting up and down 3 to 5 times. Incubate the mixture for 20 minutes at room temperature. Do not vortex or centrifuge!

### 3) Transfection

- 3.1. Add the DNA/HYPE-5 complexes dropwise into the 125mL Erlenmeyer containing cells while gently swirling the flask. Incubate the cells on orbital shaker (~125 rpm) at 37°C, 8% CO<sub>2</sub>.
- 3.2. After 4 hours of incubation, add 1mL of complete growth medium containing **320 µL of HYPE-Blast** into the 125 mL Erlenmeyer containing cells.
- 3.3. Finally, incubate your cells for 1 to 7 days depending on the type of protein expression. No medium change is required during the incubation period.

### 3. Scaling up & Scaling Down

HYPE-5™ Kit allows easy scaling up and scaling down. It achieves high protein production using different volumes and culture vessels. For scaling up or down, you will need to adjust each component in proportion to the volume of the culture medium. The Table 1 (below) shows recommended amount of HYPE-5 reagent and DNA for various volumes of the culture medium from 1 mL to 1 L. Since transfection efficiency depends on the cell model (clone, growth condition) and the culture vessels (shaker, spinner flask, bioreactor...), we recommend to perform an optimization procedure (see section 4) before starting to scale up.

**Table 1:** Suggested volume of HYPE-5 reagent, HYPE-Blast and DNA quantity according to the culture size.

Cell culture			DNA		HYPE-5 reagent		HYPE-Blast	
10 <sup>6</sup> cells per mL			1.5 µg / mL of cell culture		2µL per µg of DNA		100X dilution	
Culture volume	Culture flask	Total Cell Number*	Quantity	Dilution volume	Quantity	Dilution volume	Quantity	Dilution volume
1 mL	NA	1 x 10 <sup>6</sup>	1.5 µg	50 µL	3 µL	50µL	12 µL	100µL
30 mL	125 mL	30 x 10 <sup>6</sup>	45 µg	0.6 mL	90 µL	0.6 mL	320 µL	1 mL
250 mL	1 Liter	250 x 10 <sup>6</sup>	375 µg	5 mL	750 µL	5 mL	2.7 mL	10 mL
1 Liter	3 Liter	1000 x 10 <sup>6</sup>	1.5 mg	20 mL	3 mL	20 mL	10.8 mL	40 mL

\* The day of transfection cell density should be at 1 x 10<sup>6</sup> cells /mL

### 4. Parameters optimization

Although high protein production can be achieved in both HEK293 and CHO cell growing in suspension with the previous protocol, some optimizations may be required in order to obtain the maximum efficiency. For best results, we recommend to optimize two parameters:

- Quantity of HYPE-5 reagent and DNA
- Cell culture conditions

#### 1) HYPE-5 reagent and DNA parameters optimization:

HYPE-5 reagent must be used in slight excess compare to DNA but the optimal ratio will depend on the cell model and culture conditions.

**First step:** Maintain a fixed quantity of DNA to 1.5 µg/mL of cell culture and then vary the amount of HYPE-5 reagent from 1 to 3µL per µg of DNA (see Table 2 first step for example).

**Second step:** Once the ratio of HYPE-5 to DNA has been optimized, keep it constant and vary the DNA quantity from 1 to 2 µg per mL of cell culture (see Table 2 second step for example).

**Table 2:** Example for HYPE-5 and DNA optimization.

Step	Cell culture		DNA		HYPE-5 reagent		HYPE-Blast	
	Culture volume	Total cell Number*	Quantity µg	Dilution volume	Volume µL	Dilution volume	Quantity	Dilution volume
First step	30 mL	30 x 10 <sup>6</sup>	45	0.6 mL	45, 90, 135	0.6 mL	320 µL	1 mL
	250 mL	250 x 10 <sup>6</sup>	375	5 mL	375, 750, 1125	5 mL	2.7 mL	10 mL
Step two	30 mL	30 x 10 <sup>6</sup>	30, 45, 60	0.6 mL	Ratio from first step	0.6 mL	320 mL	1 mL
	250 mL	2.5 x 10 <sup>8</sup>	250, 375, 500	5 mL	Ratio from first step	5 mL	2.7 mL	10 mL

\* The day of transfection cell density should be at 1 x 10<sup>6</sup> cells/mL.

To test whether or not HYPE-Blast increases your protein production, we advice to use the previous optimized HYPE-5/DNA parameters in two conditions: one with and one without HYPE-Blast.

## 2) Cell culture condition optimization:

Efficient protein production is also highly dependent on the cell model. For instance, several parameters are critical to obtain the maximum efficiency such as cell suspension growth adaptation, culture medium and cell density (before and during transfection).

We recommend optimizing cell density. After setting up the best ratio of HYPE-5/DNA and the DNA quantity, test various cell densities from 0.5 to  $2 \times 10^6$  cells/mL at the time of transfection. The cells must be grown as single cells because extensive clumping at the time of transfection can reduce the quantity of protein produced. If necessary, vigorous vortexing for 10-30 seconds could be done for single cell growth recovering.

## 5. Appendix

### 5.1 Quality Controls

To guarantee the performance of each lot of HYPE-5 reagent and HYPE-Blast produced, we qualify each component using rigorous standards. The following *in vitro* assays are performed to qualify the function, quality and activity of each kit component.

Specification	Standard Quality Controls
<i>Purity</i>	Silica Gel TLC assays. Every compound shall have a single spot.
<i>Sterility</i>	Thioglycollate assay. Absence of fungal & bacterial contamination shall be obtained for 7 days.
<i>Biological Activity</i>	SEAP production yield in CHO cells growing in suspension in chemically defined medium without serum. Every batch must have an acceptance specification of > 90% of the activity of the reference lot.

### 5.2. Troubleshooting

Problem	Comments and suggestions
Low protein expression	<p>1- <b>Suboptimal transfection conditions.</b> Optimize the transfection conditions as describe in chapter 4 using a positive control plasmid.</p> <p>2- <b>Cell density.</b> A non-optimal cell density at the time of transfection can lead to low protein expression. Cells must be actively dividing at the time of transfection. About 24h before transfection pass the cells at <math>0.5-0.6 \times 10^6</math> cells/mL for having a cell density of <math>\sim 1 \times 10^6</math> cells/mL at the transfection time. However optimum cell density may depend on the cell model used and must be optimized as describe in the chapter 4.</p> <p>3- <b>Cell culture conditions.</b> 1) Cells cultured for too many passages (&gt;20-25 passages) may become resistant to transfection. Use freshly thawed cells that have been passaged at least twice. 2) Improperly cultured cells could lead to poor protein yield. Ensure complete adaptation to suspension growth conditions (medium, agitation...) to have cells growing as single and a viability &gt;90%. 3) The presence of contaminants (mycoplasma, fungi) alters considerably the transfection efficiency. Thaw a new batch of cells or use appropriate antibiotic to eliminate contamination.</p> <p>4- <b>DNA quality.</b> DNA must be highly pure, free of contaminants (proteins, phenol, ethanol etc.) and endotoxins levels must be very low since they interfere with transfection efficiencies.</p> <p>5- <b>Type of promoter.</b> Ensure that DNA promoter can be highly expressed in the cells to be transfected. Another cells or viral-driven reporter gene expression can be used as a control.</p> <p>6- <b>Medium used for preparing DNA / transfection reagent complexes.</b> It is critical that serum-free medium or buffer (HBS, PBS) are used during the preparation of the complexes. Avoid any direct contact of pure HYPE-5 reagent and pure nucleic acid solution with the plastic surface.</p> <p>7- <b>Incubation time.</b> The optimal time range between transfection and assay is generally 2-5 days. As the protein expression profile will depend on the plasmid used we recommend you to first perform a kinetic from day 1 to day 7 to evaluate the optimum incubation time.</p> <p>8- <b>Transfection reagent handling.</b> Reagents should be properly stored and must have an</p>

	ambient temperature and be vortexed prior to use.
Cells growing improperly	<p><b>1- Thawing cells are unhealthy.</b> 1) Keep cell stock in liquid nitrogen at a density of <math>1 \times 10^7</math> viable cells/mL until thawing; 2) Use low passage cells to make your own stock, 3) Use a freezing medium containing 10% DMSO and 90% of compatible medium.</p> <p><b>2- Improper culture conditions.</b> 1) Suspension cell adaptation is a key step for optimal growing cell conditions. Ensure that the culture medium is suitable for suspension growth. 2) Monitor cell density to prevent cell clumping. We suggest a 2-3 days frequency for cells passage at a density of <math>0.1-0.2 \times 10^6</math> viable cells/mL. Avoid density <math>&gt; 1.5 \times 10^6</math> viable cells/mL. 3) Monitor shaking condition to avoid toxicity, cell clumping or medium foam formation. We suggest 125 rpm with an orbital shaker as a starting point. 4) A cell culture volume of 1/3 of the total volume of the flask used is recommended.</p> <p><b>3- Concentration of HYPE-5 / nucleic acid too high.</b> Overloading the system won't systematically lead to higher protein production because too high DNA quantity (and so high HYPE-5 reagent quantity) could lead to cell toxicity. Optimize the transfection conditions as describe in chapter 4 using a positive control plasmid.</p>

Our dedicated and specialized technical support group will be pleased to answer any of your requests and to help you with your transfection experiments: [tech@ozbiosciences.com](mailto:tech@ozbiosciences.com). In addition, do not hesitate to visit our website [www.ozbiosciences.com](http://www.ozbiosciences.com) and the FAQ section.

## 6. Related Product

Description	Reference
<b>3D Transfection Technology</b>	
3D-Fect 1mL	TF21000
3D-Fectin 1mL	TN31000
<b>Magnetofection Technology</b>	
Super Magnetic Plate	MF10000
PolyMag Neo 1mL ( <i>for all nucleic acids</i> )	PG61000
CombiMag 1mL ( <i>to boost transfection reagent</i> )	CM21000
SilenceMag 1mL ( <i>for siRNA application</i> )	SM11000
NeuroMag 1mL ( <i>for transfection of neurons</i> )	NM51000
ViroMag 1mL ( <i>for all viral applications</i> )	VM41000
ViroMag R/L 1mL ( <i>for retrovirus and Lentivirus</i> )	RL41000
<b>Lipofection Technology (lipid-based)</b>	
Lullaby siRNA transfection reagent 1mL	LL71000
DreamFect Gold Transfection reagent 1mL	DG81000
FlyFectin Transfection Reagent 1mL	FF51000
<b>Protein Delivery Systems</b>	
Ab-DeliverIN 1 mL	AI21000
Pro-DeliverIN 1 mL	PI11000
<b>CaPO Transfection Kit</b>	
CP90000	
<b>Plasmids pVectOZ</b>	
pVectOZ-LacZ 25µg	PL00030
pVectOZ-SEAP 25µg	PL00050
<b>Gene &amp; Protein Tools</b>	
Bradford – Protein Assay Kit	BA00100
β-Galactosidase (CPRG) assay kits	GC10002
X-Gal Staining Kit	GX10003
<b>Biochemical</b>	
D-Luciferin, K+ 1g	LK10000
G-418, Sulfate 1g	GS21000
X-Gal powder 1g	XG11000

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. <http://www.ozbiosciences.com>.

### Limited License

The purchase of the HYPE-5™ 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). 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 HYPE-5™ 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 HYPE-5™ Transfection Kit material and documentation to OZ Biosciences, or by destroying all HYPE-5™ components. Purchasers are advised to contact OZ Biosciences with the notification that a HYPE-5™ kit is being returned 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 HYPE-5™ 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 HYPE-5™ 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  
Parc Scientifique de Luminy  
Zone Luminy Entreprise  
163, avenue de Luminy – 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](mailto:business@ozbiosciences.com)