

# HYPE-CHO™ Transfection Kit

## INSTRUCTION MANUAL



HYPE-CHO

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## Instruction manual

**HYPE-CHO™** Transfection Kit achieves **High Yield Protein Expression** in CHO cultivated in suspension.

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

**List of HYPE-CHO™ Kits**

Catalog Number	HYPE-CHO reagent	BCHO
HYC01500	1.5 ml	5 ml
HYC03000	2 x 1.5 ml	2 x 5 ml
HYC15000	15 ml	50 ml
HYC30000	2 x 15 ml	2 x 50 ml

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

Please inquire about a custom quote for bulk quantities.

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

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# 1. Introduction

## 1.1. Description

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

**HYPE-CHO™** Transfection Kit is the newest reagent dedicated to achieve **High Yield Protein Expression** in CHO cells. This kit has been designed for maximum efficiency in CHO (CHO, CHO-S, rCHO or any CHO-related cells) growing in suspension and cultivated in chemically defined medium.

This efficient transient expression system represents 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-CHO** Kit presents unique properties:

1. High Protein & Antibody production yield.
2. Suitable for 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-CHO Kit has been designed for maximum efficiency in 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 4 sizes of HYPE-CHO™ Transfection Kit:

Catalog Number	HYPE-CHO reagent	BCHO	Total volume of cell culture
HYC01500	1.5 mL	5 mL	0.5-1L
HYC03000	2x 1.5 mL	2x 5 mL	1L-2L
HYC15000	15 mL	50 mL	5-10L
HYC30000	2x 15 mL	2x 50 mL	10-20L

### Stability and Storage

Storage: Upon reception and for long-term use, store HYPE-CHO reagent and BCHO at +4°C.

Both reagents are stable for at least one year under recommended storage conditions.

Shipping condition: Room Temperature (RT)

## 1.3. Cells and Culture Medium

HYPE-CHO has been used and validated with cells from different origins (CHO, CHO-S, rCHO or any CHO-related cells in suspension). It is suitable for any kind of mammalian cell models used to produce proteins. This reagent has been tested with different chemically defined medium (FreeStyle™ CHO Expression medium, ExpiCHO...) and 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 concentration of Pluronic® surfactant (up to 0.01% w/v final concentration) to avoid dramatic impact on protein production level.

## 1.4. Important Considerations

The instructions given below represent the protocol that was applied successfully with 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 you to optimize the various parameters as described in section 4.

However, you can start by following our rapid protocol as guidelines.

The use of BCHO reagent is highly recommended yet optional. We observed, when using BCHO reagent, a large increase in protein expression with our CHO suspension cell model.

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-CHO and BCHO reagents
- 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 refer to the Table 1 in the next section. For 30 mL volume, we recommend the 125 mL flask.

Key parameters before beginning the procedure:

- DNA, HYPE-CHO and BCHO 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 0.5-2.0 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 0.8-1.2 x 10<sup>6</sup> cells/mL). Transfer 30 mL of cell culture in every 125 mL Erlenmeyer flask.

Before beginning: allow reagent to reach RT.

\*NOTE: for 1mL culture format, we recommend using a cell density of 2.5x10<sup>5</sup> cell/mL.

### 2) Complexes preparation

2.1. DNA solution. Dilute 45 to 60 µg of DNA in 600 µL of serum free medium. Incubate 5 min at RT.

2.2. HYPE-CHO solution. Dilute 90 to 120 µL of HYPE-CHO reagent in 600 µL of serum free medium. Incubate 5 min at RT.

2.3. Add the DNA solution into the HYPE-CHO 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-CHO 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. Four hours later add **320 µL of BCHO** directly 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-CHO allows easy scaling up and scaling down - it is compatible with various size volumes and culture vessels. Simply adjust each reagent proportion to the volume of culture medium. The table below shows recommended amounts of HYPE-CHO, DNA and BCHO for 1mL to 1L of cell culture medium. Since transfection efficiency is depending on the cell model (clone, growth conditions...) and the culture vessels (shaker, spinner flask, bioreactor...), we recommend performing an optimization procedure (refer to section 4.) before scaling up or down.

**Table 1: Suggested volumes of HYPE-CHO, BCHO and DNA quantity.**

Cell culture			DNA		HYPE-CHO reagent		BCHO (optional)	
10 <sup>6</sup> cells per mL			1.5 µg/mL of cell culture		2 µL per µg DNA		1X final dilution 4 h later	
Culture volume	Culture Flask	Total cell Number*	Quantity µg	Dilution volume	Volume µL	Dilution volume	Volume µL	Dilution volume
1 mL	NA	1x10 <sup>6</sup>	1.5 µg	50 µL	3 µL	50 µL	12 µL	100 µL
30 mL	125 mL	30x10 <sup>6</sup>	45 µg	0.6 mL	90 µL	0.6 mL	320 µl	1 mL
250 mL	1 Liter	250x10 <sup>6</sup>	375 µg	5 mL	750 µL	5 mL	2.7 mL	10 mL
1 Liter	3 Liter	1000x10 <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 CHO cells growing in suspension following 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-CHO reagent and DNA
- Cell culture conditions

#### 1) HYPE-CHO reagent and DNA parameters optimization

HYPE-CHO 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-CHO reagent from 1.5 to 3µL per µg of DNA (see Table 2 first step for example).

**Second step:** Once the ratio of HYPE-CHO to DNA has been optimized, keep it constant and vary the DNA quantity from 1 to 3 µg per mL of cell culture (see Table 2 second step for example).

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

Step	Cell culture		DNA		HYPE-CHO reagent		BCHO
	Culture volume	Total cell Number*	Quantity µg	Dilution volume	Volume µL	Dilution volume	Quantity
First step	30 mL	30 x 10 <sup>6</sup>	45	0.6 mL	67,90,135	0.6 mL	320 µL
	250 mL	250 x 10 <sup>6</sup>	375	5 mL	562, 750, 1125	5 mL	2.7 mL
Step two	30 mL	30 x 10 <sup>6</sup>	30, 60, 90	0.6 mL	Ratio from first step	0.6 mL	320 µL
	250 mL	2.5 x 10 <sup>8</sup>	250, 500, 750	5 mL	Ratio from first step	5 mL	2.7 mL

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

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

## 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-CHO/DNA and the DNA quantity, test various cell densities from  $0.5$  to  $2 \times 10^6$  cells/mL at the time of transfection - cells must be in their growth phase. 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-CHO reagent and BCHO 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>	Thioglycolate 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><b>1- Suboptimal transfection conditions.</b> Optimize the transfection conditions as described in chapter 4 using a positive control plasmid.</p> <p><b>2- 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><b>3- 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><b>4- 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><b>5- 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><b>6- Medium used for preparing DNA / transfection reagent complexes.</b> It is critical to use serum-free medium or buffer (HBS, PBS) during the preparation of the complexes. Avoid any direct contact of pure HYPE-CHO reagent and pure nucleic acid solution with the plastic surface.</p>

	<p><b>7- 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><b>8- Transfection reagent handling.</b> Reagents should be properly stored and must have an ambient temperature and be vortexed prior to use.</p>
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-CHO / 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-CHO 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 Products

### TRANSFECTION - Bio-Production

HYPE-5 Transfection kit - *dedicated to achieve High Yield Protein Expression in mammalian cells (CHO & CHO growing in suspension)*

### 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 application*

ViroMag - *to enhance transduction efficiency*

### PLASMIDS PVECTOZ

pVectOZ-LacZ, Luc, CAT, GFP, SEAP

### ASSAY KITS

FluoProdige – Fluorescent Protein Assay Kit

MTT cell proliferation kit

$\beta$ -Galactosidase assay kits (CPRG/ONPG)

Senescence Kit

OZBlue cell Viability Kit (Resazurin)

### BIOCHEMICALS

D-Luciferin, K<sup>+</sup> and Na<sup>+</sup>

X-Gal powder 1g / G-418, Sulfate

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.

[contact@ozbiosciences.com](mailto:contact@ozbiosciences.com) / [www.ozbiosciences.com](http://www.ozbiosciences.com)

# Purchaser Notification

## Limited License

The purchase of the HYPE-CHO™ 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.

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## Product Use Limitations

The HYPE-CHO™ 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.

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