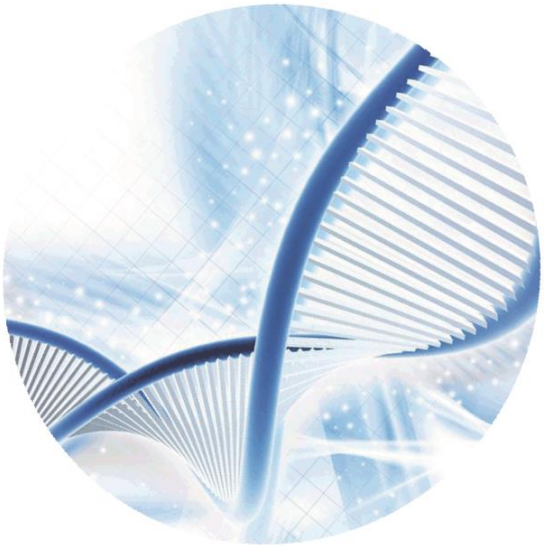


Transfection reagent

HYPE-CHO™

Transfection Kit

Achieve High Yield Protein Expression in CHO cells

Protocol

OZBIOSCIENCES
The art of delivery systems

HYPE-CHO Quick Protocol for CHO, CHOs…

To find the ideal conditions, HYPE-CHO reagent must be tested at ratios **1 µL/µg**, **2 µL/µg** and **3 µL/µg** (µL of Hype-CHO / µg of DNA). For the DNA quantity, we suggest to test from **1.5µg** to **2µg** of DNA per mL of culture medium. The BCHO reagent must be used at **1/100** of the total volume.

1

1h before transfection, dilute your cells at 1x10^6 cells per mL*

125 mL Bottle	1 L Bottle
30 mL culture medium	250 mL culture medium
30 x 10^6 cells	250 x 10^6 cells

2

Prepare 3 identical tubes of DNA

125 mL Bottle	1 L Bottle
45 or 60µg in 500 µL of serum-free medium or buffer* x3	375 or 500µg in 5mL of serum-free medium or buffer* x3

3

Prepare 3 tubes of HYPE-CHO (with 3 different amounts of reagent)

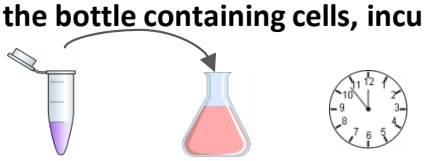
125 mL Bottle		1 L Bottle	
45µg DNA	45 µL / 90µL / 135µL in 500µL of serum-free medium or buffer*	375µg DNA	375µL/750µL/1125µL in 5mL of serum-free medium or buffer*
60µg DNA	60 µL /120µL /180µL in 500µL of serum-free medium or buffer*	500µg DNA	500µL/1000µL/1500µL in 5mL of serum-free medium or buffer*

4

Mix each DNA tube to each tube of HYPE-CHO and incubate 20min at RT*

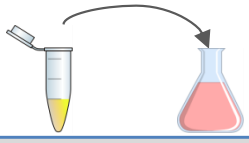
5

Distribute each mix into the bottle containing cells, incubate under orbital shaking for 4h*



6

After 4 hours, add BCHO (1/100, 1X final) reagent directly into the cells*



30 mL Culture Medium - 320µL of BCHO	250 mL Culture Medium - 2.7mL of B293

7

Incubate cells for 24h to 7 days under orbital shaking (or more if needed) at 37°C until evaluation of protein production

Choose the optimal conditions (DNA quantity, DNA/HYPE-CHO ratio, BCHO)

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ HYPE-CHO™ Kit 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 cells used to produce proteins. This Kit has been tested with several chemically defined media. 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) to avoid dramatic impact on protein production level.

- ✓ The instructions given represent protocols that were applied successfully with a variety of 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 the complete instruction manual. However, to obtain good data rapidly, 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.

- ✓ 18-24 h before transfection, seed the cells to 0.6-0.8 x 10^6 cells/mL and incubate on orbital shaker (~125 rpm) at 37°C, 8% CO2. The day of transfection, dilute the cells to 1 x 10^6 cells/mL (cell density should be about 0.8-1.2 x 10^6 cells/mL).

- ✓ Allow reagents to reach RT and gently vortex prior to use.

- ✓ **Medium or buffer without serum & supplement** must be used for the preparation of complexes (DNA/Hype-CHO). Culture media such as MEM, DMEM or OptiMEM or buffers such as HBS or PBS are recommended. In contrast, we do not recommend RPMI for preparing the complexes.

- ✓ **Formation of complexes.** 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!

- ✓ Bioreactor, spinner, flasks or Erlenmeyer etc. can be used.

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

www.ozbiosciences.com

Any questions?

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HYPE-CHO Reagent | Specifications

Package content	HYC01500: 1.5 mL of HYPE-CHO + 5 mL of BCHO HYC03000: 2 x 1.5 mL of HYPE-CHO + 2 x 5 mL of BCHO HYC15000: 15 mL of HYPE-CHO + 50 mL of BCHO HYC30000: 2 x 15 mL of HYPE-CHO + 2 x 50 mL of BCHO
Shipping conditions	Room Temperature
Storage conditions	Store the HYPE-CHO transfection reagent and BCHO at 4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product description	HYPE-CHO is a high efficiency transfection reagent specifically developed to achieve High Yield Protein Expression in CHO cells growing in suspension.
Important notice	For research use only. Not for use in diagnostic procedures

1. Cells preparation

Cell culture maintenance: sub-culture the cells at a density of 0.5-2x10^6 cells/mL for each passage (48-72h). 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 the maximum of efficiency.

18-24 h before transfection, dilute the cells to 0.6-0.8 x 10^6 cells/mL and incubate on orbital shaker (~125 rpm) at 37°C, 8% CO_2. The day of transfection, dilute the cells to 1 x 10^6 cells/mL* (cell density should be about 0.8-1.2 x 10^6 cells/mL). Transfer the volume of cells needed as described in Table 1.

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

Cell culture			DNA		HYPE-CHO reagent		BCHO (optional)	
10^6 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	1x10^6	1.5 µg	50 µL	3 µL	50 µL	12 µL	100 µL
30 mL	125 mL	30x10^6	45 µg	0.6 mL	90 µL	0.6 mL	320 µl	1 mL
250 mL	1 L	250x10^6	375 µg	5 mL	750 µL	5 mL	2.7 mL	10 mL
1 L	3 L	1x10^7	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 10^6 cells/mL.

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

2. DNA/HYPE-CHO complexes preparation

a. *HYPE-CHO*: Vortex the reagent and dilute the indicated quantity of HYPE-CHO (refer to Table 1) in 50 µL to 20 mL of culture medium without serum and supplement.

b. *DNA*: Dilute the indicated quantity of DNA (see Table 1) in 50 µL to 20 mL of culture medium without serum and supplement.

c. Add the DNA solution to the HYPE-CHO solution and mix by carefully pipetting up and down. Incubate the mix at room temperature for 20 minutes. Do not vortex or centrifuge.

3. Transfection

a. Add the HYPE-CHO / DNA complexes dropwise into cell culture bottle while gently swirling the flask to ensure a uniform distribution. Incubate the cell on orbital shaker (~125 rpm) at 37°C, 8% CO_2.

b. Four hours later add BCHO – 1X final, directly to the vessel containing cells (refer to Table 1)

c. Cultivate the cells under standard conditions for 1 to 7 days depending on the type of protein expression. No medium change is required during the incubation period.

IMPORTANT CONSIDERATIONS

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.

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 1 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 next section) before scaling up or down.

Optimization Protocol

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 step one - Table 2 first 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 step two - Table 2 step for example).

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

Table 2: Example for HYPE-CHO and DNA optimization

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

2. Cell culture conditions 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 x 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.

Additional products for bioproduction in suspension cells

- **HYPE-5** dedicated to achieve High Yield Protein Expression in mammalian cells (CHO & HEK293 growing in suspension)

- **HYPE-293** dedicated to achieve High Yield Protein Expression in HEK293 cells growing in suspension

Purchaser Notification

Limited License
The purchase of the HYPE-CHO kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). 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-CHO kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all HYPE-CHO kit reagents and documentation to OZ Biosciences, or by destroying all HYPE-CHO components. Purchasers are advised to contact OZ Biosciences with the notification that a HYPE-CHO 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 the HYPE-CHO 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
HYPE-CHO 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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