

## Quick Protocol for CHO cultivated in suspension (CHO, CHO-S ...)

To find the ideal conditions, HYPE-CHO reagent can be tested at ratios **1:1**, **2:1** and **3:1** (respectively 1  $\mu\text{L}$ , 2  $\mu\text{L}$  and 3  $\mu\text{L}$  of HYPE-CHO per  $\mu\text{g}$  of DNA). Recommended DNA quantities vary from **1.5  $\mu\text{g}$**  to **2  $\mu\text{g}$**  of DNA per mL of culture medium. BCHO reagent must be used at 1/100 of the total volume.

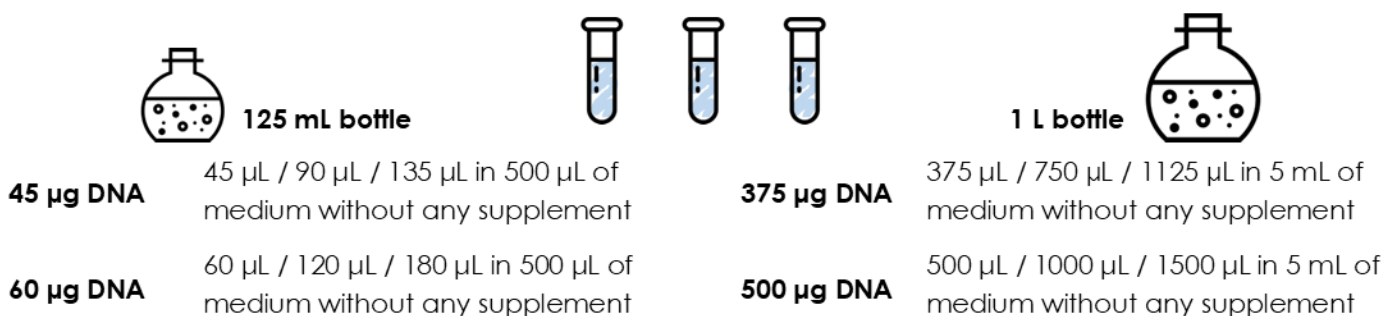
### 1. Prepare the cells



### 2. Prepare 3 identical tubes of DNA



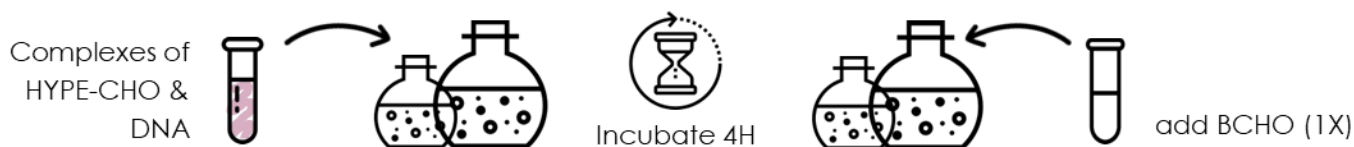
### 3. Prepare 3 tubes of HYPE-CHO corresponding to 3 different ratios



### 4. Mix each DNA suspension to each tube of HYPE-CHO dilution



### 5. Transfect cells and add BCHO reagent 4H later.



### 6. Incubate cells under orbital shaking at 37°C until evaluation of protein production

Chose the optimal conditions (DNA quantity, HYPE-CHO ratio, BCHO volume).

### Before you begin

**HYPE-CHO™** has been used and validated with cells from different origins (CHO, CHO-S, rCHO or any CHO-related cells in suspension). This Kit has been tested with several chemically defined media. 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 use of **BCHO reagent** is highly recommended yet optional.

### Protocol | DNA Transfection

#### 1. Cells preparation:

Cell culture maintenance: sub-culture the cells at a density of 0.5-2x10<sup>6</sup> cells/mL for each passage (48-72 h). Avoid high cell density and keep cell growth conditions consistent for reproducibility.

18-24 h before transfection, dilute the cells to 0.6-0.8x10<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 1x10<sup>6</sup> cells/mL (cell density should be about 1.2-1.5x10<sup>6</sup> cells/mL). Transfer the volume of cells needed as described in Table 1.

Cell Culture			DNA		HYPE-CHO		BCHO
10 <sup>6</sup> cells per mL			1.5 µg/mL		2 µl per µg DNA		1X final
Vol.	Flask.	Cell nb.*	µg	Vol.	µL	Vol.	µL
1 mL	NA	1x10 <sup>6</sup>	1.5 µg	50 µL	3 µL	50 µL	11 µL
30 mL	125 mL	30x10 <sup>6</sup>	45 µg	0.5 mL	90 µL	0.5 mL	310 µL
250 mL	1 L	250x10 <sup>6</sup>	375 µg	5 mL	750 µL	5 mL	2.6 mL
1 L	3 L	1x10 <sup>7</sup>	1.5 mg	20 mL	3 mL	20 mL	10.4 mL

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

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

#### 2. HYPE-CHO/DNA complexes preparation:

a. **HYPE-CHO:** vortex the reagent and dilute the indicated quantity of HYPE-CHO in **50 µL** to **20 mL** of culture medium without serum and supplement.

b. **DNA:** dilute the indicated quantity of DNA in **50 µL** to **20 mL** of culture medium without serum and supplement.

c. **Complexes:** add the DNA solution to the HYPE-CHO solution and mix gently by carefully pipetting up and down.

d. Incubate the mix at room temperature for **20 min.**

⚠ Do not vortex or centrifuge.

Refer to table 1 for volumes depending on size of cell culture dishes.

#### 3. Transfection

a. Add the HYPE-CHO/DNA complexes dropwise into cell culture bottle while gently swirling the flask to ensure a uniform distribution.

b. Add the BCHO reagent – **1X final** directly into the vessel containing cells.

c. Cultivate the cells under standard conditions (~**125 rpm, 37°C, 8% CO<sub>2</sub>**) for **1 to 7 days** depending on the type of protein expression. No medium change is required during the incubation period.

Our technical team is at your disposal for any questions or optimization procedures:

✉ - [tech@ozbiosciences.com](mailto: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 HYPE-CHO & BCHO:** -20°C

**Shelf life:** 1 year from the date of purchase

We recommend minimizing freeze-thaw cycles to preserve HYPE-CHO integrity.

#### Kit contents

**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

Certificate of analysis on demand.

#### Related Products

Ref	Description
#HY01500	HYPE-5, bioproduction in CHO-S & HEK-293-S
#HY29315	HYPE-293, bioproduction in 293-S
#HYV01500	HY-VIR transfection reagent for viral production

#### 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>.