

To find the ideal conditions, HYPE-5 transfection kit must be tested at ratios **1:1**, **2:1** and **3:1** (respectively 1  $\mu$ L, 2  $\mu$ L and 3  $\mu$ L of HYPE-5 per  $\mu$ g of DNA). For the DNA quantity, we suggest **45  $\mu$ g** per 30 mL, **375  $\mu$ g** per 250 mL, and **1.5 mg** per L of culture medium.

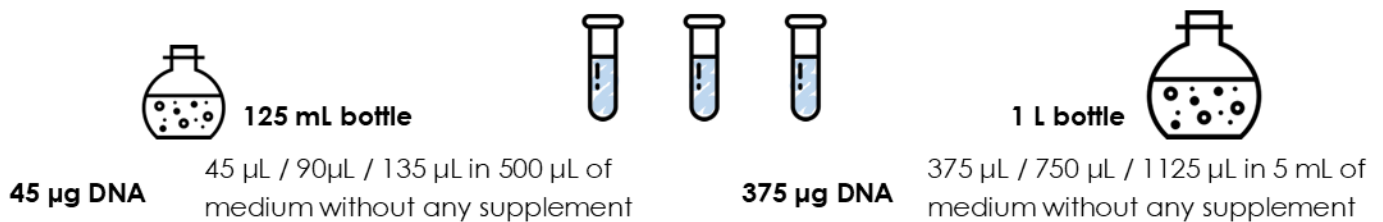
### 1. Prepare the cells



### 2. Prepare 3 identical tubes of DNA



### 3. Prepare 3 tubes of HYPE-5 corresponding to 3 different ratios



### 4. Mix each DNA suspension to each tube of HYPE-5 dilution



### 5. Transfect cells and optionally add Hype-Blast reagent 4H later.



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

Chose the optimal conditions (HYPE-5 ratio, +/- Hype-Blast).

### Before you begin

**HYPE-5™** Kit is compatible with most culture media for protein production except for CD293 from Life Technologies. 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 **HYPE-Blast** is optional for 293 cells and recommended for CHO cells.

### 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-5		HYPE-Blast	
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-5, Hype-Blast and DNA quantity

#### 2. HYPE-5/DNA complexes preparation:

a. **HYPE-5:** vortex the reagent and dilute the indicated quantity of HYPE-5 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-5 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-5/DNA complexes dropwise into cell culture bottle while gently swirling the flask to ensure a uniform distribution.

b. Optionally for CHO cells: After **4 h** of incubation, add HYPE-Blast – **1X final** into the cell culture bottle.

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-5:** -20°C | **HYPE-Blast:** +4°C

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

We recommend minimizing freeze-thaw cycles to preserve HYPE-5 integrity.

#### Kit contents

**HY01500:** 1.5 mL of HYPE-5 + 5 mL of HYPE-Blast

**HY03000:** 2 x 1.5 mL of HYPE-5 + 2 x 5 mL of HYPE-Blast

**HY15000:** 15 mL of HYPE-5 + 50 mL of HYPE-Blast

**HY30000:** 2 x 15 mL of HYPE-5 + 2 x 50 mL of HYPE-Blast

**HYR10003:** 3 mL of HYPE-5

**HYR10015:** 15 mL of HYPE-5

**HYR20030:** 2 x 15 mL of HYPE-5

**HYB00005:** 5 mL of HYPE-Blast

Certificate of analysis on demand.

#### Related Products

Ref	Description
#HY29315	HYPE-293, bioproduction in HEK-293-S
#HYC01500	HYPE-CHO, bioproduction in CHO-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>.