Transfection reagent

HYPE -5™

Transfection Kit

Achieve High Yield Protein Expression

Protocol

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Hype-5 Quick Protocol for CHOs & 293s Cells

To find the ideal conditions, Hype-5 transfection kit must be tested at ratios **1 µL/µg**, **2 µL/µg** and **3 µL/µg** (µL of Hype-5 / µg of DNA). For the DNA quantity, we suggest **45 µg** per 30mL, **375 µg** per 250mL and **1.5 mg** per L of culture medium.

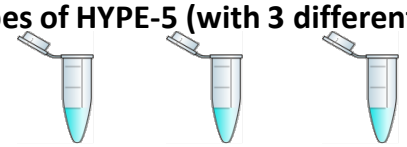
1 — **1h before transfection, dilute your cells at 1x10^6 cells per mL***

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**

125mL Bottle	1L Bottle
45µg in 600µL of serum-free medium or buffer* x 3	375µg in 5mL of serum-free medium or buffer*x 3

3 — **Prepare 3 tubes of HYPE-5 (with 3 different amounts of reagent)**



125mL Bottle	1L Bottle
45µL/90µL/135µL in 600µL of serum-free medium or buffer*	375µL/750µL/1125µL in 5mL of serum-free medium or buffer*

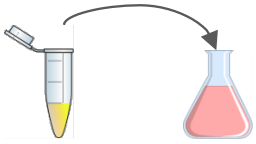
4 — **Mix each tube of DNA (step 2) to each tube of HYPE-5 (step 3) & incubate 20min at RT***

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

6 — **Prepare 3 identical tubes of HYPE-BLAST***

125mL Bottle	1L Bottle
320µL in 1mL of DMEM x3	2.7mL in 10mL of DMEM x3

7 — **Add each tube of HYPE-BLAST into the bottle containing cells, incubate under orbital shaking for 24 to 72h at 37°C until evaluation of transgene expression**



8 — **Choose the best ratio DNA:HYPE-5**

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ HYPE-5™ Kit has been used and validated with cells from different origins. 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 (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) because it could have dramatic impact on protein production level.

- ✓ The instructions given 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 at the end of this protocol. However, to obtain good data rapidly, you can start by following our quick protocol as guidelines.

- ✓ **The use of HYPE-Blast** is optional for 293 cells and recommended for CHO cells. We always recommend using the HYPE-Blast as described in the quick protocol.

- ✓ 18-24h 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% CO_2. The day of transfection, dilute the cells to 1 x 10^6 cells/mL (cell density should be about 1.2-1.5 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-5). 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-5 solution, mix gently by carefully pipetting up and down 3 to 5 times. Incubate the mixture for 20 min at room temperature. Do not vortex or centrifuge!

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

HYPE-5 Reagent | Specifications

Package content	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
Shipping conditions	Room Temperature
Storage conditions	Store the HYPE-5 transfection reagent at -20°C and HYPE-Blast at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product Descriptions	HYPE-5 is a high efficiency transfection reagent specifically developed to achieve High Yield Protein Expression in mammalian cells. This Kit has been designed for maximum recombinant protein expression in HEK293 and 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 0.05-0.2x10^6 cells/mL for each passage (48-72h). Avoid high cell density and keep cell growth conditions consistent for reproducibility.

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 1.2-1.5 x 10^6 cells/mL). Transfer the volume of cells needed as described in Table 1.

Cell culture			DNA		HYPE-5 reagent		HYPE-Blast	
10^6 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 10^6	1.5 µg	50 µL	3 µL	50µL	12 µL	100µL
30 mL	125 mL	30 x 10^6	45 µg	0.6 mL	90 µL	0.6 mL	320 µL	1 mL
250 mL	1 L	250 x 10^6	375 µg	5 mL	750 µL	5 mL	2.7 mL	10 mL
1 L	3 L	1 x 10^7	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 volume of HYPE-5 reagent, HYPE-Blast and DNA quantity according to the culture size

2. DNA/HYPE-5 complexes preparation

a. *HYPE-5*: Vortex the reagent and dilute the indicated quantity of HYPE-5 (see Table 1) in 50µL to 20 mL of culture medium without serum and supplement.

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

c. Add the DNA solution to the HYPE-5 solutions and mix gently by carefully pipetting up and down. Incubate the mix at room temperature for 20 min. Do not vortex or centrifuge.

3. Transfection

a. Add the HYPE-5 / DNA complexes dropwise into cell culture bottle while gently swirling the flask to ensure a uniform distribution. Incubate the cells on orbital shaker (~125 rpm) at 37°C, 8% CO_2.

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

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 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 described in this protocol.

Protocol | 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 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 next section) before starting to scale up.

Optimization Protocol

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 of 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).

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 10^6	45	0.6 mL	45, 90, 135	0.6 mL	320 µL	1 mL
	250 mL	250 x 10^6	375	5 mL	375, 750, 1125	5 mL	2.7 mL	10 mL
Step two	30 mL	30 x 10^6	30, 45, 60	0.6 mL	Ratio from first step	0.6 mL	320 mL	1 mL
	250 mL	2.5 x 10^8	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 10^6 cells/mL

Table 2: Example for HYPE-5 and DNA optimization

To test whether or not HYPE-Blast increases your protein production, we advise 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 x 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.

Additional products

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

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Rev. 01/2018