Transfection reagent

HYPE™ -293

Transfection Kit

Achieve High Yield Protein Expression in HEK-293 cells

Protocol

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HYPE-293 Quick Protocol for 293-F, 293s…

To find the ideal conditions, HYPE-293 reagent must be tested at ratios **1 µL/µg**, **2 µL/µg** and **3 µL/µg** (µL of Hype-293 / µg of DNA). For the DNA quantity, we suggest to test from **0.75µg** to **1.5µg** of DNA per mL of culture medium. The B293 reagent can be used at **1/100 or 1/200** of the total volume.

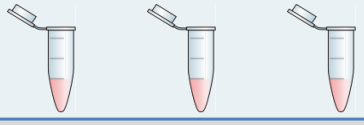
1

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
30 or 45µg in 500 µL of serum-free medium or buffer* x 3	250 or 375µg in 5mL of serum-free medium or buffer* x 3

3

Prepare 3 tubes of HYPE-293 (with 3 different amounts of reagent)

125 mL Bottle		1 L Bottle	
30µg DNA	30µL / 60µL / 90µL in 500µL of serum-free medium or buffer*	250µg DNA	250µL / 500µL / 750µL in 5mL of serum-free medium or buffer*
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*

4

Mix each DNA tube to each tube of HYPE-293 and incubate 20min at RT*

5

Add B293 reagent to each tube of complexes & immediately distribute each mix to the cells*

125 mL Bottle	1 L Bottle
150 or 300µL of B293	1.25 or 2.5mL of B293

6

Incubate cells for 24 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-293 ratio, B293)

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ HYPE-293™ Kit has been used and validated with cells from different origins (293, 293s, 293-F or any 293-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 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 instructions given represent protocols that were applied successfully with a variety of 293 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 B293 reagent** is highly recommend yet optional. We observed a large increase in protein expression with our HEK 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 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- 293). 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-293 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?

tech@ozbiosciences.com

Package content	HY29315: 1.5 mL of HYPE-293 + 5 mL of B293 HY29330: 2 x 1.5 mL of HYPE-293 + 2 x 5 mL of B293 HY293150: 15 mL of HYPE-293 + 50 mL of B293 HY293300: 2 x 15 mL of HYPE-293 + 2 x 50 mL of B293
Shipping conditions	Room Temperature
Storage conditions	Store the HYPE-293 transfection reagent and B293 at -20°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product description	HYPE-293 is a high efficiency transfection reagent specifically developed to achieve High Yield Protein Expression in HEK293 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.

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-293 reagent		B293 (optional)	
10^6 cells per mL			1.5 µg/mL of cell culture		2 µL per µg DNA		1X final dilution	
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 Liter	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-293, B293 and DNA quantity

2. DNA/HYPE-293 complexes preparation

a. *HYPE-293*: Vortex the reagent and dilute the indicated quantity of HYPE-293 (refer to 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-293 solutions and mix gently 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-293 / 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. Add the B293 reagent – 1X final (refer to Table 1) directly into the vessel containing cells.

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.

Hype-293 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-293, DNA and B293 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 the section "Protocol Optimization") before scaling up or down.

Optimization Protocol

Although high protein production can be achieved in HEK-293 cells growing in suspension following 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-293 reagent and DNA
- Cell culture conditions

1. HYPE-293 reagent and DNA parameters optimization

HYPE-293 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-293 reagent from 1 to 3µL per µg of DNA (see Step one - Table 2 for example).

Second step: Once the ratio of HYPE-293 to DNA has been optimized, keep it constant and vary the DNA quantity from 1 to 2 µg per mL of cell culture (see Step two - Table 2 for example).

Step	Cell culture		DNA		HYPE-293 reagent		B293
	Culture volume	Total cell Number*	Quantity µg	Dilution volume	Volume µL	Dilution volume	Quantity
Step two	30 mL	30 x 10^6	45	0.6 mL	45,90,135	0.6 mL	320 µL
	250 mL	250 x 10^6	375	5 mL	375, 750, 1125	5 mL	2.7 mL
Step two	30 mL	30 x 10^6	30, 45, 60	0.6 mL	Ratio from first step	0.6 mL	320 µL
	250 mL	2.5 x 10^8	250, 375, 500	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-293 and DNA optimization

To test whether or not B293 increases your protein production, we advise to use the previous optimized HYPE-293/DNA parameters in two conditions: one with and one without B293.

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-293/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

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

Purchaser Notification

Limited License
The purchase of the HYPE-293 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-293 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-293 kit reagents and documentation to OZ Biosciences, or by destroying all HYPE-293 components. Purchasers are advised to contact OZ Biosciences with the notification that a HYPE-293 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-293 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-293 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.

EUROPE & ASIA
OZ Biosciences SAS

163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
France

Ph: +33 (0) 486 948 516
Fax: +33 (0) 463 740 015

contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com

USA & CANADA
OZ Biosciences INC

7975 Dunbrook Road
Suite B
San Diego CA 92126
USA

Ph: + 1-858-246-7840
Fax: + 1-855-631-0626

contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com

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