OZBIOSCIENCES
The art of delivery systems

HYPE-293™

Quick Protocol for HEK-293 cultivated in suspension (293-S, 293-F…)

To find the ideal conditions, HYPE-293™ reagent can be tested at ratios **1:1**, **2:1** and **3:1** (respectively 1 µL, 2 µL and 3 µL of HYPE-293 per µg of DNA). Recommended DNA quantities vary from **0.75 µg** to **1.5 µg** of DNA per mL of culture medium. B293 reagent can be used at 1/100 or 1/200 of the total volume.

1. Prepare the cells

30x10⁶ cells in 30 mL

125 mL bottle

250x10⁶ cells in 250 mL

1 L bottle

1 h before transfection, dilute your cells at 1x10⁶ cells per mL

2. Prepare 3 identical tubes of DNA

125 mL bottle

30 or 45 µg DNA in 500 µL of medium without any supplement x 3

1 L bottle

250 or 375 µg DNA in 5 mL of medium without any supplement x 3

3. Prepare 3 tubes of HYPE-293 corresponding to 3 different ratios

125 mL bottle

1 L bottle

30 µg DNA	30 µL / 60 µL / 90 µL in 500 µL of medium without any supplement	**250 µg DNA**	250 µL / 500 µL / 750 µL in 5 mL of medium without any supplement
45 µg DNA	45 µL / 90µL / 135 µL in 500 µL of medium without any supplement	**375 µg DNA**	375 µL / 750 µL / 1125 µL in 5 mL of medium without any supplement

4. Mix each DNA suspension to each tube of HYPE-293 dilution

Incubate 20 min at RT

5. Transfect cells and add B293 reagent

Complexes of HYPE-293 & DNA

Optionally add B293 (recommended)

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

Chose the optimal conditions (DNA quantity, HYPE-293 ratio, B293 volume).

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HYPE-293™
Quick Protocol

Before you begin

HYPE-293™ 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 B293 reagent is highly recommended yet optional.

Protocol | DNA Transfection

1. Cells preparation:
Cell culture maintenance: sub-culture the cells at a density of 0.5-2x10^6 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 0.6-0.8x10^6 cells/mL and incubate on orbital shaker (~125 rpm) at 37 °C, 8% CO2.

The day of transfection, dilute the cells to 1x10^6 cells/mL (cell density should be about 1.2-1.5x10^6 cells/mL). Transfer the volume of cells needed as described in Table 1.

Cell Culture			DNA		HYPE-293		B293
10^6 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	1x10^6	1.5 µg	50 µL	3 µL	50 µL	11 µL
30 mL	125 mL	30x10^6	45 µg	0.5 mL	90 µL	0.5 mL	310 µL
250 mL	1 L	250x10^6	375 µg	5 mL	750 µL	5 mL	2.6 mL
1 L	3 L	1x10^7	1.5 mg	20 mL	3 mL	20 mL	10.4 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. HYPE-293/DNA complexes preparation:
a. HYPE-293: vortex the reagent and dilute the indicated quantity of HYPE-293 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-293 solution and mix gently by carefully pipetting up and down.

Incubate the mix at room temperature for 20 minutes.
Do not vortex or centrifuge.

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

3. Transfection
a. Add the HYPE-293/DNA complexes dropwise into cell culture bottle while gently swirling the flask to ensure a uniform distribution.

b. Add the B293 reagent – 1X final directly into the vessel containing cells.

c. Cultivate the cells under standard conditions (~125 rpm, 37°C, 8% CO2) 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**

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-293 & B293: -20°C

Shelf life: 1 year from the date of purchase

We recommend minimizing freeze-thaw cycles to preserve HYPE-293 integrity.

Kit contents

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

Certificate of analysis on demand.

Related Products

Ref	Description
#HY01500	HYPE-5, bioproduction in CHO-S & 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.

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