# HYPE-293™ SG

## HEK-293 Superior Grade

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

Incubate 20 min at RT

### 5. Transfect cells and add B293 SG reagent

Complexes of HYPE-293 SG & DNA

Optionally add B293 SG (recommended)

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

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

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# HYPE-293 Superior Grade
# Quick Protocol

## Before you begin

**HYPE-293 Superior Grade (SG)** 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 SG reagent** is highly recommended yet optional.

**HYPE-293 Superior Grade** is identical in synthesis and formulation to HYPE-293 Research Use Only Grade (RUO) but provides more quality controls, batch validation and documentation as required for preclinical trials.

## 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 SG | | B293 SG |
|---|---|---|---|---|---|---|---|
| 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 SG, B293 SG and DNA quantity

**2. HYPE-293 SG/DNA complexes preparation:**
*a. HYPE-293 SG:* vortex the reagent and dilute the indicated quantity of HYPE-293 SG 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 w/o serum and supplement.

*c. Complexes:* add the DNA solution to the HYPE-293 SG 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 SG/DNA complexes dropwise into cell culture bottle while gently swirling the flask to ensure a uniform distribution.

*b.* Add the B293 SG 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 SG & B293 SG:** -20°C

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

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

**Quality control:**

| | Hype 293 | Hype 293 SG |
|---|---|---|
| Sterility | ✓ | ✓ |
| Biological activity | ✓ | ✓ |
| Chemical characterization | | ✓ |
| Purity | | ✓ |
| Absence of Mycoplasma* | | ✓ |
| Endotoxin ≤ 1 EU/mL** | | ✓ |

*USP <63> Mycoplasma – **USP <85> Bacterial Endotoxin test / Certificate of analysis on demand.

## Kit contents

**HY293SG15:** 1.5 mL of HYPE-293 + 5 mL of B293
**HY293SG30:** 2 x 1.5 mL of HYPE-293 + 2 x 5 mL of B293
**HY293SG150:** 15 mL of HYPE-293 + 50 mL of B293
**HY293SG300:** 2 x 15 mL of HYPE-293 + 2 x 50 mL of B293

## Related Products

| Ref | Description |
|---|---|
| **#HY29315** | HYPE-293, bioproduction in HEK-293-S |

## 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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