Novagen®

293-Free™ Transfection Reagent

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About the Kits

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Description

293-Free™ Transfection Reagent has a unique polycationic liposomal formulation and is designed expressly for transfection of HEK293 cells grown in suspension culture. It is ideal for mammalian protein production. Derived from non-animal sources, 293-Free Transfection Reagent gives minimal cellular toxicity. It is provided as a sterile, ready-to-use solution. Benefits of this proprietary polycationic liposomal formulation include:

- Optimized for transient transfections of HEK293 suspension cultures
- Minimal cellular toxicity
- Derived from non-animal sources
- Protocol easily scales up for production
- Compatible with both serum-containing and serum-free media
- Eliminates the need for media changes

Each 1 ml of 293-Free™ Transfection Reagent is sufficient to transfect a 1-liter culture.

Storage

Store tightly capped at 4°C.

General Considerations

- Use only high quality, endotoxin-free DNA. DNA should be at a concentration of 0.5–1 µg/µl. If the DNA concentration is lower, decrease the volume of serum-free medium or PBS in the transfection protocol to compensate for the larger volume of DNA.
- Propagate and maintain cell cultures on an orbital shaker at 125 rpm at 37°C (8% CO2).
- Passage cells regularly (e.g., every 2−3 days). Avoid high density. Use only rapidly proliferating cells for transfection. To ensure reproducibility, keep cell growth conditions and density consistent.
- 293-Free™ Transfection Reagent is compatible with both serum-containing and serum-free media.

  Note: Do not include serum and antibiotics during the formation of the transfection reagent/DNA complex.

Cell Transfection Protocol

The following protocol is optimized for transfection of 25 ml HEK-293 cells in suspension in a 125-ml polycarbonate Erlenmeyer flask, however, the amounts of reagent and DNA can be varied if necessary. For other culture sizes, the reagent and DNA amounts can be scaled up or down proportionately. See Table 1 below.

Transfection Procedure

1. The day before transfection, passage HEK293 suspension cells at approximately 0.5 x 10^6 cells/ml. Incubate at ~125 rpm at 37°C (8% CO_2) overnight. The cell density should be approximately 1.0-1.5 x 10^6 cells/ml at the time of transfection.
2. On the day of transfection, dilute the cells to 1 x106 cells/ml and place 25 ml in a 125 ml shake flask.
3. Place 1 ml serum-free MEM, Opti-Pro™ SFM, or PBS into a sterile tube. Add 12.5 µg DNA. Mix.

   Note: Do not use the serum-free culture medium in which HEK293 cells were grown.
4. Add 25 µl 293-Free Transfection Reagent. Mix.
5. Incubate transfection mixture at room temperature for 15 minutes to allow 293-Free Transfection Reagent/DNA complex formation.
6. Add entire volume of transfection mixture drop wise to prepared cells.
7. Incubate cultures for 48–168 hours, at ~125 rpm at 37°C (8% CO_2).
8. Harvest cells for protein purification, characterization, or reporter assays.

Table 1. Preparation of Transfection Mixture for Various Culture Sizes

Transfection of Plasmid DNA	Culture Size			
Culture Volume (ml)	25	250	500	1000
Number of cells (x 10^6)	25	250	500	1000
Volume of MEM or PBS in the transfection mixture (ml)	1	10	20	40
Volume of 293-Free Transfection Reagent (µl)	25	250	500	1000
Amount of plasmid DNA (µg)	12.5	125	250	500