GeticoFect™ 293 Transfection Kit Instruction Manual

Product Introduction

The GeticoFect™ 293 transfection kit is specifically developed for transfecting 293 cells, designed for transient transfection in high-density culture of HEK (human embryonic kidney) 293 cells. This efficient transfection reagent and enhancer are engineered to maximize protein expression levels in 293F cells.

- Utilizes a dedicated transfection enhancer to improve transfection performance and protein expression.
- Achieves protein yields 2 to 20 times higher than other transfection reagents.
- Adopts the same transient expression protocol as low-density 293 culture systems, enabling easy transition to high-yield, high-density 293 expression systems.
- Delivers robust and reproducible transfection results, enhancing user confidence in outcomes.
- Supports scalable culture volumes from 1 mL to 1000 L while maintaining consistent protein yields.

The transfection kit consists of cationic nano GeticoFect™ 293 transfection reagent and GeticoFect™ 293 transfection enhancer. The enhancer is added 15 to 16 hours after transfection to boost transfection efficiency, cell viability, and protein expression.

Ordering Information

Catalog No.	Specification	Components	Volume
131301	1 L	GeticoFect™ 293 Kit Transfection Reagent	2.7 mL
		GeticoFect™ 293 Kit Transfection Enhancer	5 mL
131302	10 L	GeticoFect™ 293 Kit Transfection Reagent	27 mL
		GeticoFect™ 293 Kit Transfection Enhancer	50 mL
131303	50 L	GeticoFect™ 293 Kit Transfection Reagent	5 × 27 mL

Catalog No.	Specification	Components	Volume
		GeticoFect™ 293 Kit Transfection Enhancer	5 × 5 mL

Product Contents

GeticoFect™ 293 Transfection Reagent

Specifically developed and optimized for transfecting plasmid DNA into high-density 293F cells, featuring:

- Design for high-density suspension cell culture transfection, paired with an enhancer to improve transfection performance and protein yield.
- 2–20 times higher protein production than other transfection reagents in high-density 293 cell culture.
- Universal transient expression protocol for easy transition from low-density to high-yield, high-density 293 systems.
- High-quality and reproducible transfection results.
- Scalable transfection from <1 mL to >10 L with consistent protein yield.

GeticoFect™ 293 Transfection Enhancer

An optimized, chemically defined, serum-free, protein-free, and animal-component-free formulation designed to complement the 293 transfection reagent, enhancing transient transfection efficiency.

Protocol

- Gently invert GeticoFect™ 293 transfection reagent 4–5 times before use to ensure thorough mixing.
- Equilibrate plasmid DNA and GeticoFect™ 293 transfection reagent at room temperature for ~30 minutes before use.
- Incubate the GeticoFect™ 293/DNA complex for 10–20 minutes before adding to cells. Excessive incubation may slightly reduce performance.
- For 293F induced cells, blasticidin addition does not affect transfection, so no need to remove blasticidin before transfection.

Day -2: Original Cell Preparation

Prepare and culture 293F cells to a viable cell density of 3–5×10^6 cells/mL.

Day -1: Seed Cell Culture

Inoculate the 293F cells from the previous step to a density of 2.5–3×10^6 cells/mL and allow to grow overnight.

Day 0: Cell Transfection

1. Determine cell density and viability. Ensure viable cell density is 4.5–5.5×10^6 cells/mL and viability ≥95% before transfection.
2. Dilute cells to a final density of 3×10^6 cells/mL with fresh, pre-warmed 293 expression medium, then gently rotate the culture flask to mix.
3. Invert 4–5 times to ensure uniform mixing.
4. Dilute plasmid DNA with Opti-MEM I, then mix by rotation or inversion.
   Note: For most genes, the recommended final plasmid concentration is 1.0 μg/mL.
5. Dilute GeticoFect™ 293 transfection reagent with Opti-MEM I, then mix by rotation or inversion.
6. Incubate at room temperature for 5 minutes.
7. Add the diluted GeticoFect™ 293 transfection reagent to the diluted plasmid DNA, then mix by rotation or inversion.
8. Incubate at room temperature for 10–20 minutes.
9. Slowly transfer the transfection reagent complex to the cells, gently rotating the flask during addition. Culture cells in a 37°C incubator with ≥80% relative humidity and 8% CO_2 on a shaker (shaker speed see Appendix).

Day +1: Add Transfection Enhancer

1. 18–22 hours after transfection, gently add GeticoFect™ 293 transfection enhancer to the cells.

Day +5: Protein Harvest

Optimal timing depends on protein properties:

- For many secreted proteins, days 5–7 post-transfection are recommended for maximum yield.
- For membrane and intracellular proteins, days 3–4 are optimal for harvest.

Appendix 1: Transfection Reagent Dosage Calculation Table

Culture Vessel Type	96 Deep Well Plate	24 Deep Well Plate	Micro Bioreactor	125 mL Culture Flask	250 mL Culture Flask	1 L Culture Flask	2 L Culture Flask	3 L Culture Flask
Cell Count	2.0×10^6	7.5×10^6	45×10^6	75×10^6	150×10^6	600×10^6	1.2×10^9	2.25×10^9
Culture Volume	800 µL	2.5 mL	15 mL	25 mL	50 mL	200 mL	400 mL	800 mL
Shaker Speed (rpm)	900±50	225±5 250±5 235±5	240±5 250±5 245±5	125±5 (19 mm orbit) 120±5 (25 mm orbit) 95±5 (50 mm orbit)	125±5 (19 mm orbit) 120±5 (25 mm orbit) 95±5 (50 mm orbit)	125±5 (19 mm orbit) 120±5 (25 mm orbit) 95±5 (50 mm orbit)	125±5 (19 mm orbit) 120±5 (25 mm orbit) 95±5 (50 mm orbit)	90±5 90±5 55±5
Plasmid DNA Dosage	1 µg/mL final concentration (based on culture volume)	1 µg/mL final concentration (based on culture volume)	1 µg/mL final concentration (based on culture volume)	1 µg/mL final concentration (based on culture volume)	1 µg/mL final concentration (based on culture volume)	1 µg/mL final concentration (based on culture volume)	1 µg/mL final concentration (based on culture volume)	1 µg/mL final concentration (based on culture volume)
Plasmid DNA Volume	0.8 µL	2.5 µL	15 µL	25 µL	50 µL	200 µL	400 µL	800 µL
Opti-MEM I Medium	50 µL	150 µL	900 µL	1.5 mL	3 mL	12 mL	24 mL	48 mL
GeticoFect™ 293 Transfection Reagent	2.5 µL	8 µL	50 µL	80 µL	160 µL	640 µL	1.3 mL	2.6 mL

Culture Vessel Type	96 Deep Well Plate	24 Deep Well Plate	Micro Bioreactor	125 mL Culture Flask	250 mL Culture Flask	1 L Culture Flask	2 L Culture Flask	3 L Culture Flask
Opti-MEM I Medium	50 µL	140 µL	850 µL	1.4 mL	2.8 mL	11.2 mL	22.5 mL	45 mL
GeticoFect™ 293 Transfection Enhancer	5 µL	15 µL	90 µL	150 µL	300 µL	1.2 mL	2.4 mL	4.8 mL
Final Medium Volume	~1 mL	~3 mL	~20 mL	~30 mL	~60 mL	~40 mL	~480 mL	~960 mL