



# GeticoFect™ CHO Transfection Kit User Manual

## Instruction Manual

### Ordering Information

Catalog No.	Specification	Components	Volume
131801	1 L	GeticoFect™ CHO Transfection Reagent	3.2 mL
		GeticoFect™ CHO Transfection Enhancer	6 mL
131802	10 L	GeticoFect™ CHO Transfection Reagent	32 mL
		GeticoFect™ CHO Transfection Enhancer	60 mL
131803	50 L	GeticoFect™ CHO Transfection Reagent	5 × 32 mL
		GeticoFect™ CHO Transfection Enhancer	5 × 60 mL

## Protocol

### Transfection Guidelines

To achieve greater flexibility, the CHO expression system provides three different expression protocols:

- **Standard protocol:** After adding GeticoFect™ CHO enhancer 1 day post-transfection, culture cells at 37°C.
- **High-titer protocol:** After adding the enhancer 1 day post-transfection, culture cells at 32°C.
- **Max-titer protocol:** After adding the enhancer 1 day post-transfection, culture cells at 32°C.



## Transfecting GeticoFect™ CHO-S Cells

- Gently rotate to mix cells during all procedures; avoid vigorous mixing/pipetting. Cell viability and health are critical.

1. Passage GeticoFect™ CHO-S cells until the cell density reaches approximately  $4 \times 10^6$ – $6 \times 10^6$  cells/mL.

### Day –1: Cell Pretreatment

2. One day before transfection, dilute GeticoFect CHO-S cells to a final density of  $3 \times 10^6$ – $4 \times 10^6$  viable cells/mL and allow to grow overnight.

### Day 0: Transfecting Cells

3. On day 0, measure viable cell density and viability. Proceed with transfection only if the density is  $\sim 7 \times 10^6$ – $10 \times 10^6$  viable cells/mL and viability is 95–99%.
4. Dilute the cells from step 2 to a final density of  $6 \times 10^6$  viable cells/mL with fresh, pre-warmed (37°C) CHO expression medium, and gently rotate the flask to mix.
5. Pre-mix GeticoFect™ CHO transfection/plasmid DNA complex and store at 4°C, following these steps:
  - a) Gently invert GeticoFect™ CHO transfection reagent 4–5 times to mix.
  - b) Dilute plasmid DNA with OptiPRO medium at 4°C and invert to mix.
  - c) Dilute GeticoFect™ CHO transfection reagent with OptiPRO medium at 4°C and invert to mix.
  - d) Add the diluted transfection reagent to the diluted plasmid DNA and invert to mix.
6. Incubate the GeticoFect™ CHO transfection reagent/plasmid DNA complex at room temperature for 1–5 minutes, then slowly transfer to the cells in step 4 and shake gently to mix.
7. Place the flask on a shaker at 37°C, 8% CO<sub>2</sub>, with shaking speed as per the appendix table.

### Day 1: Adding GeticoFect™ CHO Enhancer

18–22 hours after transfection, select one protocol to add the reagent:

- **Standard protocol:** Add the appropriate amount of GeticoFect™ CHO enhancer (see appendix table), gently rotate the flask, and return to 37°C, 8% CO<sub>2</sub> for culture.
- **High-titer protocol:** Add the enhancer, gently rotate the flask, and return to 32°C, 5% CO<sub>2</sub> for culture.
- **Max-titer protocol:** Add the enhancer, gently rotate the flask, and return to 37°C, 5% CO<sub>2</sub> for culture.

### Protein Harvesting

- Standard protocol: 8–10 days post-transfection.
- High-titer protocol: 10–12 days post-transfection.
- Max-titer protocol: 12–14 days post-transfection.

**Cell Culture Calculation Table**

Flask Volume	125 mL	250 mL	500 mL	1 L	2 L	3 L
Cell Count	1.5×10 <sup>8</sup>	3.0×10 <sup>8</sup>	6.0×10 <sup>8</sup>	1.2×10 <sup>9</sup>	2.4×10 <sup>9</sup>	4.5×10 <sup>9</sup>
Transfection Culture Volume	25 mL	50 mL	100 mL	200 mL	400 mL	750 mL
Shaker Speed (rpm)	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)	125±5 (19 mm orbit) 120±5 (25 mm orbit) 95±5 (50 mm orbit)	75±5 80±5 80±5
Flask Type	Ventilated flask with baffle	Ventilated flask with baffle	Ventilated flask with baffle	Ventilated flask with baffle	Ventilated flask with baffle	Ventilated flask with baffle
DNA Dosage	0.5–1.0 µg/mL final concentration	0.5–1.0 µg/mL final concentration	0.5–1.0 µg/mL final concentration	0.5–1.0 µg/mL final concentration	0.5–1.0 µg/mL final concentration	0.5–1.0 µg/mL final concentration
Plasmid DNA Volume	20 µL	40 µL	80 µL	160 µL	320 µL	600 µL
OptiPRO SFM Medium for DNA Dilution	1 mL	2 mL	4 mL	8 mL	16 mL	30 mL
GeticoFect™ CHO Transfection Reagent	80 µL	160 µL	320 µL	640 µL	1280 µL	2400 µL



Flask Volume	125 mL	250 mL	500 mL	1 L	2 L	3 L
OptiPRO SFM Medium for Reagent Dilution	920 mL	1.84 mL	3.7 mL	7.4 mL	14.8 mL	28 mL
GeticoFect™ CHO Enhancer	150 µL	300 µL	600 µL	1200 µL	2400 µL	4500 µL
Final Culture Volume	~35 mL	~70 mL	~140 mL	~280 mL	~560 mL	~1 L