



GeticoFect™ EasyStyle Plus CHO Expression Kit

User Manual

Instruction Manual

I. Experimental Preparation

(A) Reagents and Materials Preparation

System Components: Prepare components based on experimental needs, verify quantity and quality, and check for damage or deterioration:

1. EasyStyle CHO-S® cells: Stored in liquid nitrogen. Each vial contains 1×10^7 cells in 90% EasyStyle CHO expression medium + 10% DMSO.
2. EasyStyle PLUS reagent: Store at 4°C (do not freeze). 1 mL suffices for 25 transfections + 1 control (37.5 µL per transfection), composition confidential.
3. EasyStyle CHO expression medium: Store at 4°C protected from light. Add L-glutamine to 8 mM before use (e.g., 40 mL of 200 mM stock per 1 L medium). 1 L bottle, serum-free, chemically defined, composition confidential.
4. OptiPRO™ SFM: Store at 4°C protected from light. 100 mL bottle, serum-free, promotes DNA-lipid complex formation, composition confidential.
5. pCMV SPORT-βgal: Store at -20°C. 25 µg (0.5 µg/µL), positive control for transfection and expression.

Consumables and Equipment:

1. Sterile consumables: 125-mL/250-mL/500-mL/1L/3L disposable sterile vented-cap polycarbonate conical flasks (VWR or Corning, part numbers in document); sterile pipettes, tips; cryovials.
2. Instruments: Liquid nitrogen tank; 37°C water bath; CO₂ incubator (8% humidity, 8% CO₂); orbital shaker (120–135 rpm for culture, 70–80 rpm for large-scale transfection); centrifuge (100×g); cell counter (e.g., Coulter Counter) or hemocytometer; vortex mixer; biosafety cabinet.

(B) Experimental Environment Preparation



1. Perform all operations in a biosafety cabinet. Sterilize with UV light for ≥ 30 minutes before use; ensure a clean, sterile work surface.
2. Maintain a tidy work area to avoid cross-contamination. Pre-calibrate instruments to ensure normal operation.

II. Cell Culture

(A) Thawing and Establishing Culture

1. **Preparations:** Remove EasyStyle CHO-S[®] cryovial from liquid nitrogen; pre-warm EasyStyle CHO expression medium (with 8 mM L-glutamine) in 37°C water bath; prepare 125-mL sterile vented flask; set incubator shaker parameters (37°C, 8% CO₂, 125 rpm).
2. **Thaw cells:** Quickly thaw vial in 37°C water bath, shaking gently. When only small ice crystals remain, remove, disinfect with 70% ethanol.
3. **Inoculate cells:** Transfer cell suspension to 125-mL flask with 30 mL pre-warmed medium, mix gently.
4. **Culture and observe:** Incubate on shaker. Next day, test viability/density via Trypan Blue staining. Continue if viability >70%; thaw new vial if <60%.
5. **First passage:** 24–48 hours post-thaw, passage at 0.3×10^6 viable cells/mL in pre-warmed medium. Use 125-mL or 250-mL flasks with 40 mL or 80 mL cell suspension. Passage ≥ 5 times before transfection.

(B) Cell Passaging

1. **Routine passage (48–72 hours):**
 1. Count cells: When density reaches $1\text{--}1.5 \times 10^6$ viable cells/mL, test viability/density via Trypan Blue.
 2. Calculate and dilute: Dilute to $0.5\text{--}2 \times 10^5$ viable cells/mL with pre-warmed medium, transfer to appropriate flask.
 3. Culture: Incubate at 37°C, 8% CO₂, 120–135 rpm.
2. **Daily passage:**
 1. When density reaches 2×10^6 viable cells/mL, passage to 0.5×10^6 cells/mL (or $0.8\text{--}1.0 \times 10^6$ cells/mL for expansion).
 2. Next day, check density; continue daily passage to maintain below upper limit for transfection efficiency.
3. **Large-scale culture:** Inoculate at 0.5×10^6 viable cells/mL. Harvest at 5×10^6 cells/mL (3–4 days); do not overgrow. If clumping occurs, vortex 10–30 seconds to disperse.

(C) Cell Cryopreservation

1. **Prepare cryopreservation medium:** 0.9 mL fresh medium (8 mM L-glutamine) + 0.1 mL DMSO per 1 mL, filter-sterilize, keep on ice.
2. **Prepare cells:** Culture to $\geq 1 \times 10^7$ viable cells/mL, transfer to centrifuge tube, test viability/density.



3. **Centrifuge and resuspend:** Centrifuge at 100×g, room temperature, 5 min. Aspirate supernatant, resuspend in pre-chilled medium to 1×10^7 cells/mL.
4. **Aliquot and freeze:** Dispense 1 mL per cryovial, use programmed freezer ($-1^\circ\text{C}/\text{min}$), then store in liquid nitrogen. Test recovery 24 hours later.

III. Cell Transfection

(A) Plasmid Preparation

Extract plasmid DNA using a kit, ensuring sterility, no phenol/sodium chloride contamination. Filter through 0.22 μm membrane, adjust to 1 mg/mL.

(B) Transfection Preparation

1. Prepare log-phase EasyStyle CHO-S® cells with viability >95%. Calculate cell volume based on transfection scale.
2. Ready EasyStyle PLUS reagent, OptiPRO™ SFM, pre-warmed (37°C) EasyStyle CHO medium (8 mM L-glutamine), 125-mL flasks, vortex, shaker, incubator.

(C) Transfection Protocol (30 mL Example)

1. **Cell preparation:** 24 hours before transfection, passage at $5\text{--}6 \times 10^5$ cells/mL. On transfection day, check density ($1.2\text{--}1.5 \times 10^6$ cells/mL); adjust to 1×10^6 cells/mL, transfer 30 mL to 125-mL flask.
2. **Reagent preparation:** Invert EasyStyle PLUS reagent tube to mix (no vortex). Dilute 37.5 μg plasmid DNA and 37.5 μL reagent each to 0.6 mL with OptiPRO™ SFM, invert to mix.
3. **Complex formation:** Add diluted reagent to diluted DNA (total 1.2 mL), mix gently, incubate 10 min (≤ 20 min) at room temperature.
4. **Transfect cells:** Add complex to flask, rotate gently to disperse. Incubate at 37°C , 8% CO_2 , 135 rpm; no medium change for 6–7 days.

(D) Transfection Optimization and Scaling

1. **Optimize expression:** Perform time-course experiments (1–7 days post-transfection) to monitor protein yield and viability. Test plasmid DNA (30–45 μg) and reagent (30–45 μL) doses. For GFP reporters, monitor from 24 hours, but assess protein yield alongside transfection efficiency.
2. **Scale up:** Increase reagent volumes proportionally for larger cultures.

IV. Result Detection and Analysis

(A) β -Galactosidase Activity Assay

1. Harvest cells at specified time points, prepare lysates per FluoReporter® lacZ/galactosidase kit instructions.
2. Add substrate, react under optimal conditions, measure activity via microplate reader.

(B) Target Protein Detection

1. Select methods (Western blot, ELISA, etc.) based on protein properties.

2. Determine optimal expression time, collect supernatant/lysates for quantification.

V. Troubleshooting

(A) Cell Culture Issues

Problem	Cause	Solution
No viable cells after thawing	Improper storage; faulty cryovials; wrong thaw medium; incorrect shaker settings	Order new cryovials, store in liquid nitrogen; use 1×10^7 cells/mL for cryopreservation; use 90% fresh medium + 10% DMSO; freeze low-passage cells; thaw in medium with 8 mM L-glutamine, no antibiotics; set shaker to 37°C, 8% CO ₂ , 125 rpm
Slow growth	Wrong medium; excessive antibiotics; incorrect shaker settings; foaming medium; inappropriate flask size; high passage number	Use medium with 8 mM L-glutamine; limit antibiotics to ≤ 5 mL/L 0.5X Pen-Strep; correct shaker settings; reduce speed to eliminate foam; use flasks $\geq 2.5 \times$ culture volume; use cells $\leq$ passage 25
Cell clumping	Inadequate mixing; irregular passaging; improper density	Increase mixing; establish regular passaging schedule; maintain recommended density

(B) Transfection and Protein Production Issues

Problem	Cause	Solution
Low transfection efficiency and/or protein yield	High passage number; no passage before transfection; poor cell culture; excessive antibiotics; improper reagent use; poor plasmid DNA quality; suboptimal conditions; DNA contamination; toxic gene; wrong harvest time	Thaw low-passage cells; passage at $5-6 \times 10^5$ cells/mL 24 hours before transfection; follow culture protocol strictly; reduce antibiotics; store/use reagent correctly; avoid miniprep DNA, use low-endotoxin kit; optimize with positive control, adjust DNA/reagent doses; ensure DNA sterility; switch expression system; perform time-course to find optimal harvest