Gxpress CHO Transfection & Medium Kit II

for CHO Cells "Gene Expression System"

Description

Gxpress CHO Transfection & Medium Kit II has been developed for the gene expression system of Chinese hamster ovary (CHO) cells, such as CHO-K1 or CHO-S cells, and the high production of recombinant proteins or the transfection assay in serum-free culture. All contents of Gxpress CHO Transfection & Medium Kit II are a chemically defined composition that no contains serum, protein, animal origin, hydrolysates, and no unknown composition. (Storage; 2°C to 8°C / Protect from light)

Package Contents (Catalog Number GXCHO-MK-0010)

• Gxpress CHO Transfection Kit*	(Catalog Number GXCHO-RK-0010)
*Gxpress CHO TF Reagent	2.4 mL (1.2 mL x 2)
*CHO Complexing Solution	80 mL (40 mL x 2)
*Gxpress CHO Enhancer	120 mL
• CH400 medium (1,000 mL)	(Catalog Number CH400-0010)

Important Points

- Subculture CHO cells a minimum of three times to allow them to recover from thawing before use.
- Plasmid DNA must be clean.
- Gently mix Transfection Reagent and DNA into CHO Complexing Solution.
- Use CHO Complexing Solution in transfection assay.

Culture Conditions

- Cell line: CHO cells
- Culture type: Suspension
- Culture vessels: Flask or culture bag, etc.
- Incubate atmosphere: Humidified atmosphere of 5–8% CO₂ in air
- Temperature range: 36°C to 38°C
- Shaker culture: 120–130 rpm

Required Materials

Plasmid DNA

- Erlenmeyer shaker flask, vent-cap
- CHO-S or CHO-K1 cells
- CO₂ Incubator and Shaker

Gxpress CHO TF Reagent

Gxpress CHO TF Reagent is a cationic lipid-based reagent for transferring DNA into mammalian cells. Gently mix Reagent by pipetting it up and down before use. Use CHO Complexing Solution in transfection assay. Gently mix Reagent and DNA into CHO Complexing Solution.

CH400 medium

CH400 medium has been developed for the growth of CHO cells and the high production of recombinant proteins or the transfection assay in serum-free culture. Medium contains no L-alanyl-L-glutamine and requires supplementation with Lglutamine or L-alanyl-L-glutamine before use. Medium contains no HT and antibiotics. Please supply to the medium as necessary.

	Schedule	Steps	Procedures
Day -1 (Preculture)	1	Seeding of CHO cells	Transfer 6 x 10^7 viable cells into 125-mL flask containing 30 mL of CH400 medium. (a seeding density of 2 × 10^6 cells/mL)
	2 12	Shaker culture	Temperature : 37° , Shaker speed : 125 rpm, Atmosphere : $5-8\%$ CO ₂ in air.
Day 0 (Transfection)	3	Preparation of cells	Determine viable cell density. The density should be $4-6 \times 10^6$ cells/mL (> 95% viability). Add 5 x 10 ⁷ cells into 125-mL flask containing 25 mL of CH400 medium. (a seeding density of 2 × 10 ⁶ cells/mL)
	4 CHO Complexing Solution	Prepare DNA mixtures	Dilute 30 μ g of plasmid DNA in 1.2 mL of CHO Complexing Solution. Mix gently by inversion one time.
	CHO Complexing Solution	Prepare Gxpress CHO TF Reagent mixtures	Dilute 72 μ L of Gxpress CHO TF Reagent in 1.2 mL of CHO Complexing Solution. Mix gently by inversion one time. Incubate for 5 min at room temperature.
	$\begin{array}{c} 6 \\ Reagent \\ DNA \\ Complexes \\ \end{array}$	Prepare DNA/Reagent complexes	After 5 min incubation, add diluted Gxpress CHO TF Reagent to diluted DNA . Mix gently by inversion one time. Incubate for 5 min at room temperature. Note; Do not mix vigorously.
	7 Complexes	Add DNA/Reagent to CHO cells	After 5 min incubation, transfer gently the complexes to CHO cells. Note; Longer incubation times may result in decrease expression.
	8 771	Shaker culture	Temperature : 37° , Shaker speed : 125 rpm, Atmosphere : $5-8\%$ CO ₂ in air.
Day 1 (Enhancer)	9 Enhancer	Add Gxpress CHO Enhancer to CHO cells	After 18 – 24 h transfection, add 3 mL of Gxpress CHO Enhancer to CHO cells.
	10 71	Shaker culture	Temperature : 37° C, Shaker speed : 125 rpm, Atmosphere : $5-8\%$ CO ₂ in air.
Day 2 – 7 (Expression)		Harvest cells or media	Evaluate recombinant protein expression. Culture time for optimal protein expression depends on the nature of recombinant protein.

Gxpress CHO TF Reagent Transfection Protocol (30 mL Scale)