# **Gxpress CHO Transfection & Medium Kit**

## for CHO Cells "Gene Expression System"

## **Description**

Gxpress CHO Transfection & Medium Kit 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 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-MAK-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

• CH400AZ medium (1,000 mL) (Catalog Number CH400AZ-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% CO<sub>2</sub> 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
- CO<sub>2</sub> 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.

### CH400AZ medium

CH400AZ 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 L-alanyl-L-glutamine and dose not require supplementation with L-glutamine or L-alanyl-L-glutamine. Medium contains no HT and antibiotics. Please supply to the medium as necessary.

# Gxpress CHO TF Reagent Transfection Protocol (30 mL Scale)

	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 CH400AZ medium. (a seeding density of 2 $\times$ $10^6$ cells/mL)
	2 //	Shaker culture	Temperature : $37^{\circ}$ C, Shaker speed : $125$ rpm, Atmosphere : $5-8\%$ CO <sub>2</sub> 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 $5 \times 10^7$ cells into 125-mL flask containing 25 mL of CH400AZ medium. (a seeding density of $2 \times 10^6$ cells/mL)
	4 CHO Complexing Solution	Prepare DNA mixtures	Dilute 30 µg of plasmid DNA in 1.2 mL of CHO Complexing Solution. Mix gently by inversion one time.
	Reagent  CHO Complexing Solution	Prepare Gxpress CHO TF Reagent mixtures	Dilute 72 $\mu$ 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.
	6	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 (7)	Shaker culture	Temperature : $37^{\circ}$ C, Shaker speed : $125$ rpm, Atmosphere : $5-8\%$ CO <sub>2</sub> 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 (7)	Shaker culture	Temperature : $37^{\circ}$ C, Shaker speed : $125$ rpm, Atmosphere : $5-8\%$ CO <sub>2</sub> in air.
Day 2 — 7 (Expression)	11 1 1	Harvest cells or media	Evaluate recombinant protein expression.  Culture time for optimal protein expression depends on the nature of recombinant protein.