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℃ to 8℃ / 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% CO_2 in air
Temperature range: 36℃ to 38℃
Shaker culture: 120–130 rpm

Required Materials

- Plasmid DNA
- Erlenmeyer shaker flask, vent-cap
- CHO-S or CHO-K1 cells
- CO_2 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 L-glutamine or L-alanyl-L-glutamine before use. Medium contains no hypoxanthine, thymidine, and antibiotics. Please supply to the medium as necessary.

＜ Transfection Kit Method ＞

Thaw CHO cells and Subculture method

For Shaker Culture (125-mL Shaker Flasks)

1 Thaw CHO cells in a water bath and transfer into a 15-mL tube containing 10 mL of CH400 medium.
2 Resuspend with 10 mL of CH400 medium, count cells and determine cell viability.
3 Transfer cells at a seeding density of 2 x 10^5 cells/mL into a 125-mL shaker flask containing 30 mL of CH400 medium and incubate at 37℃ in shaker culture (125 rpm).
4 On the second day culture, harvest cells with a 50-mL tube, and determine the viable cell density.
5 Transfer cells at a seeding density of 2 x 10^5 cells/mL into a 125-mL shaker flask containing 30 mL of CH400 medium and incubate at 37℃.
6 On the fourth day culture, harvest cells with a 50-mL tube, and determine the viable cell density.
7 Subculture cells at a seeding density of 2 x 10^5 cells/mL every 4 days with fresh CH400 medium.
8 For your experiments before using, subculture CHO cells a minimum of three times to allow them to recover from cryopreservation. The interval of subculture is performed at 3 days interval when glutamine is selected, and at 4 days interval when selecting alanyl-glutamine.

Set-up CHO cells into flasks

For Shaker Culture (125-mL Shaker Flasks)

1 Transfer cells at a seeding density of 1–2 x 10^6 cells/mL into a 125-mL shaker flask containing 30 mL of CH400 medium and incubate at 37℃ in shaker culture (125 rpm).
2 On the first day culture, harvest cells with a 50-mL tube, and determine the viable cell density.
3 Resuspend cells in log-phase growth (>95% viability) with CH400 medium.
4 Transfer cells at a seeding density of 2 x 10^6 cells/mL into a 125-mL shaker flask containing 25 mL of CH400 medium and incubate at 37℃.

Transfection assay

For Shaker Culture (125-mL Shaker Flasks)

1 Dilute 30 µg of plasmid DNA in 1.2 mL of CHO Complexing Solution. Mix gently by inversion one time.
2 Dilute 72 µL 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.
3 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. Excessive mixing may result in decrease expression.**
4 After 5 min incubation, transfer gently the DNA/Reagent complexes to CHO cells into 125-mL flask. **Note; Longer incubation times may result in decrease expression.**
5 Incubate cells at 37℃ in shaker culture (125 rpm).
6 After 18-24 h incubation, add 3 mL of Gxpress CHO Enhancer to CHO cells and incubate at 37℃.
7 Harvest cells or media to evaluate recombinant protein expression. Culture time for optimal protein expression depends on the nature of recombinant protein.

Scaling up transfection assay

Culture	Size	12-well plate	125-mL flask	1-L flask	2-L flask	3-L flask
	Volume	1 mL	25 mL	200 mL	400 mL	800 mL
	Cell Number	2×10^6	5×10^7	4×10^8	8×10^8	1.6×10^9
	Cell Density	2.0×10^6 cells/mL				
	Shaker Speed	125 ± 5 rpm				90 ± 5 rpm
DNA	DNA Conc.	1 µg	30 µg	240 µg	480 µg	960 µg
	CHO Complexing Solution	40 µL	1.2 mL	9.6 mL	19.2 mL	38.4 mL
Reagent	Gxpress CHO TF Reagent	2.4 µL	72 µL	576 µL	1,152 µL	2,304 µL
	CHO Complexing Solution	40 µL	1.2 mL	9.6 mL	19.2 mL	38.4 mL
Enhancer	Gxpress CHO Enhancer	100 µL	3 mL	24 mL	48 mL	96 mL

*Shaker speed should be determined empirically based on the specific laboratory used.
*Volume of CHO Complexing Solution used to dilute plasmid DNA and Gxpress CHO TF Reagent.

Optimization for transfection assay

Transfection condition for CHO cells can optimize by varying the amount of Gxpress CHO TF Reagent used with 30 µg plasmid DNA.

● CH400 medium volume: 25 mL

- Plasmid DNA amounts: 30 µg
- Total cell numbers: 3–9 x 10^7 cells (1–3 x 10^6 cells/mL, > 95% viability)
- Gxpress CHO TF Reagent amounts: 50–90 µL (e.g., 54, 63, 72, 81, 90 µL)
- CHO Complexing Solution volume: each 1.2 mL x 2 (total 2.4 mL)
- Gxpress CHO Enhancer: 3 mL

Cryopreservation

1 Prepare the cryopreservation medium of 90% CH400 medium and 10% DMSO.
2 Harvest cells and resuspend at a cell density of 5–10 x 10^6 cells/mL with the fresh cryopreservation medium.
3 Transfer CHO cells into cryovials.
4 Achieve cryopreservation following standard procedures, do not directly put into liquid nitrogen.
5 Transfer frozen cells to liquid nitrogen.

Other information

For Research Use Only. Not for use in diagnostic procedures. This product is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Related product

＜ Transfection System ＞

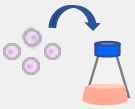
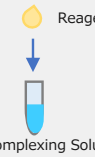
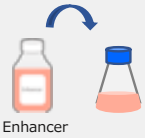
Gxpress CHO Transfection & Medium Kit	GXCHO-MAK-0010
Gxpress CHO Transfection & Medium Kit II	GXCHO-MK-0010
Gxpress CHO Transfection Kit	GXCHO-RK-0010
Gxpress CHO TF Reagent	GXCHO-TF-0010
Gxpress CHO Enhancer	GXCHO-EN-0010

＜ Chemically Defined Medium ＞

CH100 medium	CH100-0010	Adhesive culture
CH150 medium	CH150-0005	Cloning assay
CH200 medium	CH200-0010	Suspension culture
CH300 medium	CH300-0010	Suspension culture
CH400 medium	CH400-0010	Suspension culture
CH400AZ medium*	CH400AZ-0010	Suspension culture
Gxpress CHO Feed medium	GXCHO-FD-0010	Fed-Batch culture
Scattering reagent CHO	SRCHO-005	Anti-clumping reagent

* Ready-to-use medium with L-alanyl-L-glutamine

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 CH400 medium. (a seeding density of 2 × 10^6 cells/mL)
	2 	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 x 10^6 cells/mL (> 95% viability). Add 5 x 10^7 cells into 125-mL flask containing 25 mL of CH400AZ medium. (a seeding density of 2 × 10^6 cells/mL)
	4 	Prepare DNA mixtures	Dilute 30 µg of plasmid DNA in 1.2 mL of CHO Complexing Solution. Mix gently by inversion one time.
	5 	Prepare Gxpress CHO TF Reagent mixtures	Dilute 72 µL 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 	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 	Shaker culture	Temperature : 37℃, Shaker speed : 125 rpm, Atmosphere : 5−8% CO_2 in air.
Day 1 (Enhancer)	9 	Add Gxpress CHO Enhancer to CHO cells	After 18 – 24 h transfection, add 3 mL of Gxpress CHO Enhancer to CHO cells.
	10 	Shaker culture	Temperature : 37℃, Shaker speed : 125 rpm, Atmosphere : 5−8% CO_2 in air.
Day 2 – 7 (Expression)	11 	Harvest cells or media	Evaluate recombinant protein expression. Culture time for optimal protein expression depends on the nature of recombinant protein.