Gxpress 293 Transfection & Medium Kit II

for 293 Cells “Gene Expression System”

Description

Gxpress 293 Transfection & Medium Kit II has been developed for the gene expression system of human embryonic kidney (HEK) 293 cells, such as Expi293F, 293-F, HEK293, 293T, or VPC cells, and the high production of recombinant proteins or the transfection assay in serum-free culture. All contents of Gxpress 293 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 GX293-MK-0010）

● Gxpress 293 Transfection Kit*	(Catalog Number GX293-RK-0010)
*Gxpress 293 TF Reagent	2.8 mL (1.4 mL x 2)
*293 Complexing Solution	100 mL (50 mL x 2)
*Gxpress 293 Enhancer	60 mL
● HE400 medium (1,000 mL)	(Catalog Number HE400-0010)

Important Points

- Subculture 293 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 293 Complexing Solution.
- Use 293 Complexing Solution in transfection assay.

Culture Conditions

Cell line: 293 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
- Expi293F or 293T cells
- CO_2 Incubator and Shaker

Gxpress 293 TF Reagent

Gxpress 293 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 293 Complexing Solution in transfection assay. Gently mix Reagent and DNA into 293 Complexing Solution.

HE400 medium

HE400 medium has been developed for the growth of 293 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 antibiotics. Please supply to the medium as necessary.

＜ Transfection Kit Method ＞

Thaw 293 cells and Subculture method

For Shaker Culture (125-mL Shaker Flasks)

1 Thaw 293 cells in a water bath and transfer into a 15-mL tube containing 10 mL of HE400 medium.
2 Resuspend with 10 mL of HE400 medium, count cells and determine cell viability.
3 Transfer cells at a seeding density of 3 x 10^5 cells/mL into a 125-mL shaker flask containing 30 mL of HE400 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 3 x 10^5 cells/mL into a 125-mL shaker flask containing 30 mL of HE400 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 3 x 10^5 cells/mL every 4 days with fresh HE400 medium.
8 For your experiments before using, subculture 293 cells a minimum of three times to allow them to recover from cryopreservation.

Set-up 293 cells into flasks

For Shaker Culture (125-mL Shaker Flasks)

1 Transfer cells at a seeding density of 2.5–3 x 10^6 cells/mL into a 125-mL shaker flask containing 30 mL of HE400 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 HE400 medium.
4 Transfer cells at a seeding density of 3 x 10^6 cells/mL into a 125-mL shaker flask containing 25 mL of HE400 medium and incubate at 37℃.

Transfection assay

For Shaker Culture (125-mL Shaker Flasks)

1 Dilute 30 µg of plasmid DNA in 1.5 mL of 293 Complexing Solution. Mix gently by inversion one time.
2 Dilute 84 µL of Gxpress 293 TF Reagent in 1.5 mL of 293 Complexing Solution. Mix gently by inversion one time. Incubate for 5 min at room temperature.
3 After 5 min incubation, add diluted Gxpress 293 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 293 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 1.5 mL of Gxpress 293 Enhancer to 293 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	3×10^6	7.5×10^7	6×10^8	1.2×10^9	2.4×10^9
	Cell Density	3.0×10^6 cells/mL				
	Shaker Speed	125 ± 5 rpm				90 ± 5 rpm
DNA	DNA Conc.	1 µg	30 µg	240 µg	480 µg	960 µg
	293 Complexing Solution	50 µL	1.5 mL	12 mL	24 mL	48 mL
Reagent	Gxpress 293 TF Reagent	2.8 µL	84 µL	672 µL	1,344 µL	2,688 µL
	293 Complexing Solution	50 µL	1.5 mL	12 mL	24 mL	48 mL
Enhancer	Gxpress 293 Enhancer	50 µL	1.5 mL	12 mL	24 mL	48 mL

*Shaker speed should be determined empirically based on the specific laboratory used.
*Volume of 293 Complexing Solution used to dilute plasmid DNA and Gxpress 293 TF Reagent.

Optimization for transfection assay

Transfection condition for 293 cells can optimize by varying the amount of Gxpress 293 TF Reagent used with 30 µg plasmid DNA.

● HE400 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 293 TF Reagent amounts: 70–100 µL (e.g., 72, 78, 84, 90, 96 µL)
- 293 Complexing Solution volume: each 1.5 mL x 2 (total 3.0 mL)
- Gxpress 293 Enhancer: 1.5 mL

Cryopreservation

1 Prepare the cryopreservation medium of 90% HE400 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 293 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 ＞

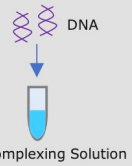
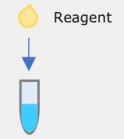
Gxpress 293 Transfection & Medium Kit	GX293-MAK-0010
Gxpress 293 Transfection & Medium Kit II	GX293-MK-0010
Gxpress 293 Transfection Kit	GX293-RK-0010
Gxpress 293 TF Reagent	GX293-TF-0010
Gxpress 293 Enhancer	GX293-EN-0010

＜ Chemically Defined Medium ＞

HE100 medium	HE100-0010	Adhesive culture
HE150 medium	HE150-0005	Cloning assay
HE200 medium	HE200-0010	Suspension culture
HE300 medium	HE300-0010	Suspension culture
HE300AZ medium*	HE300AZ-0010	Suspension culture
HE400 medium	HE400-0010	Suspension culture
HE400AZ medium*	HE400AZ-0010	Suspension culture
Gxpress 293 Feed medium	GX293-FD-0010	Fed-Batch culture

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

Gxpress 293 TF Reagent Transfection Protocol (30 mL Scale)

Schedule		Steps	Procedures
Day -1 (Preculture)	1 	Seeding of 293 cells	Transfer 9 x 10^7 viable cells into 125-mL flask containing 30 mL of HE400 medium. (a seeding density of 3 × 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 3 – 5 x 10^6 cells/mL (> 95% viability). Add 7.5 x 10^7 cells into 125-mL flask containing 25 mL of HE400 medium. (a seeding density of 3 × 10^6 cells/mL)
	4 	Prepare DNA mixtures	Dilute 30 µg of plasmid DNA in 1.5 mL of 293 Complexing Solution. Mix gently by inversion one time.
	5 	Prepare Gxpress 293 TF Reagent mixtures	Dilute 84 µL of Gxpress 293 TF Reagent in 1.5 mL of 293 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 293 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 293 cells	After 5 min incubation, transfer gently the complexes to 293 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 293 Enhancer to 293 cells	After 18 – 24 h transfection, add 1.5 mL of Gxpress 293 Enhancer to 293 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.