

# Gxpress 293 Feed medium

## Chemically defined medium

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### Description

Gxpress 293 Feed medium has been developed for the growth and productivity of human embryonic kidney (HEK) 293 cells, such as Expi293F, 293-F, 293T, or VPC cells. Gxpress 293 Feed medium demonstrates that the capability for increasing cell growth and production of recombinant proteins in serum-free culture. Gxpress 293 Feed medium is a chemically defined, serum-free, protein-free, animal origin-free medium that contains no protein, hydrolysates, or components of unknown composition.

(Storage; 2°C to 8°C / Protect from light)

### Culture conditions

Cell line: 293 cells

Culture type: Suspension or Adhesive

Culture vessels: Flask, plate, dish, 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

### Reconstitute Gxpress 293 Feed medium (Powder)

- 1 Add Gxpress 293 Feed medium at 62.5 g/L to ultra-pure water (20°C to 35°C) at 85% final volume.
- 2 Dissolved with stirrer at room temperature. Do not heat water.
- 3 Add 1N NaOH at 45 mL/L and mix.
- 4 Add NaHCO<sub>3</sub> at 3.0 g/L and mix.
- 5 Adjust the pH of the medium with 1N NaOH to 7.2 to 7.6.
- 6 Add ultra-pure water to final volume.
- 7 Filter the medium through 0.2 µm or less.

### Thaw 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 HE400AZ medium.
- 2 Resuspend with 10 mL of HE400AZ medium, count cells and determine cell viability.
- 3 Transfer cells at a seeding density of 3 x 10<sup>5</sup> cells/mL (2–4 x 10<sup>5</sup> cells/mL) into a 125-mL shaker flask containing 30 mL of HE400AZ medium and incubate at 37°C and 120–130 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  $3 \times 10^5$  cells/mL ( $2\text{--}4 \times 10^5$  cells/mL) into a 125-mL shaker flask containing 30 mL of HE400AZ medium and incubate at 37°C.
- 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  $3 \times 10^5$  cells/mL ( $2\text{--}4 \times 10^5$  cells/mL) every 4 days (3–5 days) with fresh HE400AZ medium.
- 8 For your experiments before using, subculture 293 cells a minimum of three times to allow them to recover from cryopreservation.

### Supplementation method

The variables to consider when designing an optimized nutrient supplementation strategy are timing and quantity of the Feed medium to add to the culture. In general, adding up to a total 5–40% over the life of the culture can yield improved protein production. Gxpress 293 Feed medium contains no L-glutamine, L-alanyl-L-glutamine, or antibiotics.

#### For Single-Day Supplementation (125-mL Shaker Flasks)

- 1 Harvest cells and determine the viable cell density.
- 2 Ensure that the growth rate is in mid-logarithmic phase.
- 3 Transfer cells at a seeding density of  $2\text{--}3 \times 10^5$  cells/mL into a 125-mL shaker flask containing 25–30 mL of HE400AZ medium and incubate at 37°C and 120–130 rpm.
- 4 On the first day culture, add Gxpress 293 Feed medium at 5%, 10%, 20%, 30%, or 40% volume of supplementation into the culture medium.
- 5 Determine cell densities and amounts of recombinant proteins.

#### For Multiple-Day Supplementation (125-mL Shaker Flasks)

- 1 Harvest cells and determine the viable cell density.
- 2 Ensure that the growth rate is in mid-logarithmic phase.
- 3 Transfer cells at a seeding density of  $2\text{--}3 \times 10^5$  cells/mL into a 125-mL shaker flask containing 25–30 mL of HE400AZ medium and incubate at 37°C and 120–130 rpm.
- 4 Add the Feed medium at 5% volume on Day 0 or Day 1 culture.
- 5 Add the Feed medium at 5% volume on Day 2 or Day 3 culture.
- 6 Add the Feed medium at 5% volume on Day 4 or Day 5 culture.
- 7 Add the Feed medium at 5% volume on Day 6 or Day 7 culture.
- 8 Add the Feed medium at 5% volume on Day 8 or Day 9 culture.
- 9 Determine cell densities and amounts of recombinant proteins.

### Glucose, Glutamine, and Others Supplementation

Glucose and Glutamine may rapidly deplete in 293 cell cultures. It may be advantageous to addition with a concentrate of them, either empirically determined or based on monitoring of culture medium. Also, it

may be possible to quantifying all amino acids to construct a supplement of those components that have become depleted in another option.

### 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