

HE550 medium

Chemically defined medium

Description

HE550 medium has been developed to support the growth of human embryonic kidney (HEK) 293 cells, including Expi293F Pro, Expi293F, 293-F, 293T, and VPC cells, and to enable high-level production of recombinant proteins or transfection assays under serum-free culture conditions. HE550 medium is a chemically defined, serum-free, protein-free, and animal origin-free medium that contains no proteins, hydrolysates, or components of unknown composition.

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

Culture conditions

Cell line: 293 cells

Culture type: Suspension

Culture vessels: Flask, plate, dish, 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

Prepare medium

HE550 medium contains L-alanyl-L-glutamine and does not require supplementation with L-glutamine or additional L-alanyl-L-glutamine.

HE550 medium contains no antibiotics. Please supplement with antibiotics as necessary.

Thaw and Subculture method

For Shaker Culture (125-mL Shaker Flasks)

- 1 Thaw 293 cells in a 37 °C water bath and transfer them into a 15-mL tube containing 10 mL of HE550 medium.
- 2 Centrifuge if necessary, discard the supernatant, and resuspend the cells in 10 mL of fresh HE550 medium. Count the cells and determine cell viability.
- 3 Seed the cells at a density of 5×10^5 cells/mL (range: $3\text{--}6 \times 10^5$ cells/mL) into a 125-mL shaker flask containing 30 mL of HE550 medium. Incubate at 37 °C with appropriate shaking conditions.
- 4 On day 2 of culture, collect the cells into a 50-mL tube and determine the viable cell density.
- 5 Reseed the cells at 5×10^5 cells/mL (range: $3\text{--}6 \times 10^5$ cells/mL) in a 125-mL shaker flask containing 30 mL of fresh HE550 medium and incubate at 37 °C.
- 6 On day 4 of culture after reseeding, collect the cells into a 50-mL tube and determine the viable cell

density.

- 7 Subculture the cells every 3–5 days (typically every 4 days) at a seeding density of 5×10^5 cells/mL (range: $3\text{--}6 \times 10^5$ cells/mL) using fresh HE550 medium.
- 8 Before experimental use, maintain the 293 cells for at least 2–3 weeks to allow full recovery from cryopreservation.
- 9 HE550 medium supports subculture without overgrowth up to a viable cell density of approximately 0.8×10^7 cells/mL. Subculture is recommended every 3–4 days.

Adaptation to HE550 medium

293 cells previously maintained in other serum-free media can be adapted to HE550 medium by repeated subculture using the procedure described above. The recommended seeding density is 5×10^5 cells/mL (range: $3\text{--}6 \times 10^5$ cells/mL). Lower seeding densities may result in suboptimal cell growth. 293 cells maintained in serum-containing medium can be adapted to HE550 medium through either direct or sequential adaptation. First, continue the adaptation process under the same culture conditions currently in use (e.g., adherent culture or suspension culture).

Cryopreservation

Collect 293 cells in the mid-log growth phase with a viability of $\geq 95\%$ by centrifugation ($200 \times g$ for 5 minutes). Resuspend the cell pellet in freezing medium (HE550 medium supplemented with 10% DMSO) at a final cell density of $5\text{--}10 \times 10^6$ cells/mL.

Freeze the cells using a standard controlled-rate freezing method and store them in liquid nitrogen.

To ensure efficient recovery after thawing, cryopreservation at low cell densities is not recommended.

Other information

For Research Use Only. Not for use in diagnostic procedures.

This product is intended for research and development purposes only. It is not intended for human or animal therapeutic or clinical diagnostic use. This product must not be used for food, drug, household, agricultural, or cosmetic applications.

Please read the Safety Data Sheet (SDS) carefully before use and follow all recommended handling instructions. Appropriate personal protective equipment, including protective eyewear, clothing, and gloves, should be worn when handling this product.

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
HE550 medium	HE550-0010	Suspension culture
HE550AZ medium*	HE550AZ-0010	Suspension culture
Gxpress 293 Feed medium	GX293-FD-0010	Fed-Batch culture

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