HE400AZ medium

Chemically defined medium

Description

HE400AZ medium has been developed for the growth of human embryonic kidney (HEK) 293 cells, such as Expi293F, 293-F, or 293T cells, and the high production of recombinant proteins or the transfection assay in serum-free culture. HE400AZ 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℃ to 8℃ / 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% CO_2 in air
Temperature range: 36℃ to 38℃
Shaker culture: 120–130 rpm

Prepare medium

HE400AZ medium contains L-alanyl-L-glutamine and dose not require supplementation with L-glutamine or L-alanyl-L-glutamine.

1 HE400AZ medium contains no antibiotics. Please supply to the medium as necessary.

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 10^5 cells/mL (2–4 x 10^5 cells/mL) into a 125-mL shaker flask containing 30 mL of HE400AZ medium and incubate at 37℃.
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 (2–4 x 10^5 cells/mL) into a 125-mL shaker flask containing 30 mL of HE400AZ 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 (2–4 x 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.

For Static Culture (T75 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 10^5 cells/mL (2–4 x 10^5 cells/mL) into a T75 flask containing 25 mL of HE400AZ medium and incubate at 37℃.
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 (2–4 x 10^5 cells/mL) into a T75 flask containing 25 mL of HE400AZ 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 (2–4 x 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.

Adaptation method to HE400AZ medium

We recommend both direct and sequential adaptation method, adapting 293 cells to HE400AZ medium. It is critical that the growth rate is in mid-logarithmic phase before adaptation culture.

For direct adaptation (125-mL Shaker Flasks)

1 Harvest cells and ensure that the growth rate is in mid-logarithmic phase.
2 Transfer cells at a seeding density of 3 x 10^5 cells/mL (2–4 x 10^5 cells/mL) into a 125-mL shaker flask containing 30 mL of HE400AZ medium and incubate at 37℃.
3 On the fourth day culture, harvest cells by pipetting with a 50-mL tube and determine the viable cell density. **Do not use trypsin.**
4 Subculture cells at a seeding density of 3 x 10^5 cells/mL (2–4 x 10^5 cells/mL) every 4 days (3–5 days) with fresh HE400AZ medium.
5 Continue to subculture cells as necessary every 4 days (3–5 days) with fresh HE400AZ medium until consistent growth is achieved.

Cryopreservation

1 Prepare the cryopreservation medium of 90% HE400AZ 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