

HE150 medium

Chemically defined medium

Description

HE150 medium has been developed for the single cell cloning assay of human embryonic kidney (HEK) 293 cells, such as Expi293F, 293-F, 293T, or VPC cells, in serum-free culture. HE150 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: 96-well plate, cloning plate, etc.

Incubate atmosphere: Humidified atmosphere of 5–8% CO₂ in air

Temperature range: 36°C to 38°C

Prepare medium

HE150 medium requires supplementation with L-glutamine or L-alanyl-L-glutamine.

- 1 Add 200 mM L-glutamine or L-alanyl-L-glutamine, 2–8 mM final concentration, to the medium.
- 2 HE150 medium contains no antibiotics. Please supply to the medium as necessary.

Thaw cells and subculture method

For Shaker Culture (125-mL Shaker Flask)

- 1 Thaw 293 cells in a water bath and transfer into a 15-mL tube containing 10 mL of subculture medium.
- 2 Resuspend with 10 mL of medium, count cells and determine cell viability.
- 3 Transfer cells at a seeding density of 3×10^5 cells/mL ($2\text{--}4 \times 10^5$ cells/mL) into a 125-mL shaker flask containing 30 mL of subculture medium and incubate at 37°C in shaker culture (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 3×10^5 cells/mL ($2\text{--}4 \times 10^5$ cells/mL) into a 125-mL shaker flask containing 30 mL of subculture 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 3×10^5 cells/mL ($2\text{--}4 \times 10^5$ cells/mL) every 4 days (3–5 days) with fresh subculture medium.
- 8 For your experiments before using, subculture 293 cells a minimum of three times to allow them to recover from cryopreservation.

Single cell cloning assay

For Static Culture (96-well plate)

- 1 Transfer cells at a seeding density of 3×10^5 cells/mL ($2\text{--}4 \times 10^5$ cells/mL) into a 125-mL shaker flask containing 30 mL of subculture medium and incubate at 37°C in shaker culture (120–130 rpm).
- 2 On the second 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 HE150 medium.
- 4 Centrifuge cells once more to pellets and resuspend with HE150 medium. **Note: Important to replace HE150 medium, not included the subculture medium.**
- 5 Dilute cells to a final density of 0.5–5 viable cells/well with HE150 medium.
- 6 Transfer cells into 96-well plate (0.2 mL/well) and incubate at 37°C.
- 7 Observe cell clones into plates every day and estimate each cell clone.

Expansion culture

For Static Culture (from 96-well plate to 100-mm dish)

- 1 Prepare the required volume of HE150/subculture medium (50:50 ratio of HE150 medium to subculture medium) before expansion culture.
- 2 Harvest cell clones by pipetting. **Do not use trypsin to prevent cell damage and death.**
- 3 Transfer cells into a 24-well plate containing 1.5 mL of HE150/subculture medium and incubate at 37°C in static culture.
- 4 On the 2–4 days culture, harvest and transfer cells into a 6-well plate containing 5 mL of HE150/subculture medium.
- 5 On the 2–4 days culture, harvest and transfer cells into a 100-mm dish containing 20 mL of HE150/subculture medium.

For Shaker Culture (from 100-mm dish to 125-mL shaker flask)

- 1 On the 2–4 days culture, harvest cells with a 50-mL tube, resuspend with 10 mL of subculture medium, count cells, and determine the viable cell density.
- 2 Transfer cells at a seeding density of 3×10^5 cells/mL ($2\text{--}4 \times 10^5$ cells/mL) into a 125-mL shaker flask containing 30 mL of subculture medium and incubate at 37°C in shaker culture (120–130 rpm).
- 3 On the second day culture, harvest cells with a 50-mL tube, and determine the viable cell density.
- 4 Transfer cells at a seeding density of 3×10^5 cells/mL ($2\text{--}4 \times 10^5$ cells/mL) into a 125-mL shaker flask containing 30 mL of subculture medium and incubate at 37°C.
- 5 On the fourth day culture, harvest cells with a 50-mL tube, and determine the viable cell density.
- 6 Subculture cells at a seeding density of 3×10^5 cells/mL ($2\text{--}4 \times 10^5$ cells/mL) every 4 days (3–5 days) with fresh subculture medium.
- 7 Continue to subculture cells as necessary every 4 days with fresh subculture medium until consistent growth is achieved.

Cryopreservation

- 1 Prepare the cryopreservation medium of 90% subculture medium and 10% DMSO.
- 2 Harvest cells and resuspend at a cell density of 5–10 x 10⁶ 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 >

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|-------------------------|---------------|--------------------|
| 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