CH150 medium

Chemically defined medium

Description

CH150 medium has been developed for the single cell cloning assay of Chinese hamster ovary (CHO) cells, such as CHO-S, CHO-K1, DG44, or DXB11 cells, in serum-free culture. CH150 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: CHO cells
Culture type: Suspension or Adhesive
Culture vessels: 96-well plate, cloning plate, etc.
Incubate atmosphere: Humidified atmosphere of 5–8% CO_2 in air
Temperature range: 36℃ to 38℃

Prepare medium

CH150 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 CH150 medium contains no hypoxanthine, thymidine, and antibiotics. Please supply to the medium as necessary.

Thaw cells and subculture method

For Shaker Culture (125-mL Shaker Flask)

1 Thaw CHO 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 2 x 10^5 cells/mL (1–3 x 10^5 cells/mL) into a 125-mL shaker flask containing 30 mL of subculture medium and incubate at 37℃ 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 2 x 10^5 cells/mL (1–3 x 10^5 cells/mL) into a 125-mL shaker flask containing 30 mL of subculture medium and incubate at 37℃.
6 On the third day culture, harvest cells with a 50-mL tube, and determine the viable cell density.
7 Subculture cells at a seeding density of 2 x 10^5 cells/mL (1–3 x 10^5 cells/mL) every 3 days (2–4 days) with fresh subculture medium.
8 For your experiments before using, subculture CHO 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 2 x 10^5 cells/mL (1–3 x 10^5 cells/mL) into a 125-mL shaker flask containing 30 mL of subculture medium and incubate at 37℃ 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 CH150 medium.
4 Centrifuge cells once more to pellets and resuspend with CH150 medium. **Note: Important to replace CH150 medium, not included the subculture medium.**
5 Dilute cells to a final density of 0.5–5 viable cells/well with CH150 medium.
6 Transfer cells into 96-well plate (0.2 mL/well) and incubate at 37℃.
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 CH150/subculture medium (50:50 ratio of CH150 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 CH150/subculture medium and incubate at 37℃ in static culture.
4 On the 2–3 days culture, harvest and transfer cells into a 6-well plate containing 5 mL of CH150/subculture medium.
5 On the 2–3 days culture, harvest and transfer cells into a 100-mm dish containing 20 mL of CH150/subculture medium.

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

1 On the 2–3 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 2 x 10^5 cells/mL (1–3 x 10^5 cells/mL) into a 125-mL shaker flask containing 30 mL of subculture medium and incubate at 37℃ 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 2 x 10^5 cells/mL (1–3 x 10^5 cells/mL) into a 125-mL shaker flask containing 30 mL of subculture medium and incubate at 37℃.
5 On the third day culture, harvest cells with a 50-mL tube, and determine the viable cell density.
6 Subculture cells at a seeding density of 2 x 10^5 cells/mL (1–3 x 10^5 cells/mL) every 3 days (2–4 days) with fresh subculture medium.
7 Continue to subculture cells as necessary every 3 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 10^6 cells/mL with the fresh cryopreservation medium.
3 Transfer CHO 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 CHO Transfection & Medium Kit	GXCHO-MAK-0010
Gxpress CHO Transfection & Medium Kit II	GXCHO-MK-0010
Gxpress CHO Transfection Kit	GXCHO-RK-0010
Gxpress CHO TF Reagent	GXCHO-TF-0010
Gxpress CHO Enhancer	GXCHO-EN-0010

＜ Chemically Defined Medium ＞

CH100 medium	CH100-0010	Adhesive culture
CH150 medium	CH150-0005	Cloning assay
CH200 medium	CH200-0010	Suspension culture
CH300 medium	CH300-0010	Suspension culture
CH300AZ medium*	CH300AZ-0010	Suspension culture
CH400 medium	CH400-0010	Suspension culture
CH400AZ medium*	CH400AZ-0010	Suspension culture
Gxpress CHO Feed medium	GXCHO-FD-0010	Fed-Batch culture
Scattering reagent CHO	SRCHO-005	Anti-clumping reagent

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