CH300 medium

Chemically defined medium

Description

CH300 medium has been developed for the growth of Chinese hamster ovary (CHO) cells, such as CHO-S, CHO-K1, DG44, or DXB11 cells, and the high production of recombinant proteins or the transfection assay in serum-free culture. CH300 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
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

CH300 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 CH300 medium contains no hypoxanthine, thymidine, and antibiotics. Please supply to the medium as necessary.

Thaw and Subculture method

For Shaker Culture (125-mL Shaker Flasks)

1 Add 200 mM L-glutamine (8 mM final concentration, 40 mL/L) to the medium before use.
2 Thaw CHO cells in a water bath and transfer into a 15-mL tube containing 10 mL of CH300 medium.
3 Resuspend with 10 mL of CH300 medium, count cells and determine cell viability.
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 CH300 medium and incubate at 37℃.
5 On the second day culture, harvest cells with a 50-mL tube, and determine the viable cell density.
6 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 CH300 medium and incubate at 37℃.
7 On the third day culture, harvest cells with a 50-mL tube, and determine the viable cell density.
8 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 CH300 medium.

9 For your experiments before using, subculture CHO cells a minimum of three times to allow them to recover from cryopreservation.

For Static Culture (T75 Flasks)

1 Add 200 mM L-glutamine (8 mM final concentration, 40 mL/L) to the medium before use.
2 Thaw CHO cells in a water bath and transfer into a 15-mL tube containing 10 mL of CH300 medium.
3 Resuspend with 10 mL of CH300 medium, count cells and determine cell viability.
4 Transfer cells at a seeding density of 2 x 10^5 cells/mL (1–3 x 10^5 cells/mL) into a T75 flask containing 25 mL of CH300 medium and incubate at 37℃.
5 On the second day culture, harvest cells with a 50-mL tube, and determine the viable cell density.
6 Transfer cells at a seeding density of 2 x 10^5 cells/mL (1–3 x 10^5 cells/mL) into a T75 flask containing 25 mL of CH300 medium and incubate at 37℃.
7 On the third day culture, harvest cells with a 50-mL tube, and determine the viable cell density.
8 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 CH300 medium.
9 For your experiments before using, subculture CHO cells a minimum of three times to allow them to recover from cryopreservation.

Adaptation method to CH300 medium

We recommend both direct and sequential adaptation method, adapting CHO cells to CH300 medium. It is critical that the growth rate is in mid-logarithmic phase before adaptation culture.

For direct adaptation (125-mL Shaker Flasks)

1 Add 200 mM L-glutamine (8 mM final concentration, 40 mL/L) to the medium before use.
2 Harvest cells and ensure that the growth rate is in mid-logarithmic phase.
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 CH300 medium and incubate at 37℃.
4 On the third day culture, harvest cells by pipetting with a 50-mL tube and determine the viable cell density. **Do not use trypsin.**
5 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 CH300 medium.
6 Continue to subculture cells as necessary every 3 days (2–4 days) with fresh CH300 medium until consistent growth is achieved.

Cryopreservation

1 Prepare the cryopreservation medium of 90% CH300 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