GIBCO
invitrogen cell culture

CD CHO Medium

GIBCO® CD CHO Medium is developed for the growth of Chinese Hamster Ovary (CHO) cells and expression of recombinant proteins in suspension culture. CD CHO is an animal origin-free, chemically defined medium that contains no proteins, hydrolysates, or components of unknown composition.

Description	Cat. No.	Size
CD CHO Medium	**10743-011** **10743-029**	**500mL** **1000mL**
CD CHO Medium – AGT™ ADVANCED GRANULATION TECHNOLOGY	**12490-017** **12490-025** **12490-001** **12490-003**	**1 x L** **10 x L** **100 x L** **10 Kg**

Intended Use

For research use/further cell culture manufacturing. CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

Precautions

CD CHO Medium requires supplementation of 200mM L-glutamine or GLUTAMAX™-I prior to use.

For Glutamine Synthetase Expression Systems that do not require L-glutamine supplementation, CD CHO Medium may require additional supplementation (i.e. GS Supplement 50X, Cat. No. 0010020DG).

Lower levels of L-glutamine should be considered if using a fed batch or perfusion protocol or if the cell line in use is sensitive to ammonia.

CD CHO Medium is made **without** hypoxanthine and thymidine for use in dihydrofolate reductase (DHFR) amplified systems; for other applications, add 10 ml/L of HT Supplement (Cat. No. 11067) prior to use.

Formulated without phenol red to minimize estrogen-like effects of phenol red.

Storage

Liquid: 2 to 8°C Protect from light

AGT: 2 to 8°C Store Dark and Dry

Shelf Life

Liquid: 12 months

AGT: 24 months

Physical Conditions

Standard physical conditions for CHO cells grown in CD CHO complete medium are 36 to 38°C in a humidified atmosphere of 8 to 10% CO_2 in air. Using standard aseptic conditions, cultures may be grown in shake flasks (35 mL cell suspension in 125 mL shake flask) on an orbital shaker platform rotating at 125 to 135 rpm or in spinner flasks (rpm may vary with impeller design). Loosen caps of flasks to permit gas exchange (vented caps can also be used). Avoid overexposure of cultures to light.

Medium Preparation

CD CHO requires supplementation of GLUTAMAX-I or 200mM L-glutamine. Aseptically add 8 mM (40mL/L) to the medium before use.

The addition of a surfactant such as PLURONIC®. F-68 is not required.

If L-glutamine is not required, add 40 mL of sterile distilled water and adjust the osmolality to 320 mOsm using a sterile solution of NaCl.

Reconstitution of CD CHO AGT:

1. Measure 90% of final volume distilled water (Cat No. 15230).
2. Add CD CHO Medium AGT to water. Mix for 30 minutes or until dissolved completely.
3. Dilute to final volume with water.
4. Sterilize by membrane filtration.
5. Store at 2-8°C. Protect from light
6. Aseptically supplement with GLUTAMAX-I or L-glutamine at time of use.

Notes: CD CHO Medium AGT contains sodium bicarbonate. **DO NOT ADD additional** sodium bicarbonate**.**

CD CHO Medium AGT is auto pH and osmolality adjusted, no further adjustment required. For final lot pH and osmolality specifications please refer to Certificate of Analysis specification.

Recovery

1. Rapidly thaw (< 1 minute) frozen vial in a 37°C water bath.
2. Triturate and transfer the entire contents of the cryovial (1.5 x 10^7 cells) into a 125 mL shake flask containing 28.5 mL of pre-warmed CD CHO supplemented with 8mM GLUTAMAX-I or L-glutamine.
3. Incubate at 36-38°C in a humidified atmosphere of 8 -10% CO_2 in air on an orbital shaker platform rotating at 125 to 135 rpm. Loosen caps of flasks to allow for gas exchange.
4. Subculture cells 3-5 days post thaw at a seeding density of 3 x 10^5 viable cells/mL. It is recommended to subculture

cells for a minimum of 3 passages before use in other applications.

Note: Do not centrifuge the cells as they are extremely fragile upon recovery from cryopreservation.

Subculturing CHO Cells in CD CHO Medium

Sequential adaptation of CHO cells from serum supplemented or serum-free medium may be required. **It is critical that cell viability be at least 90% and cells be in the mid-logarithmic phase of growth prior to adaptation.** The recommended sequential adaptation procedure is as follows:

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Sequential Adaptation:

1. Subculture CHO cells grown in conventional medium with 5-10% serum or other serum-free medium into a 50:50 ratio of CD CHO to the original media. During the adaptation procedure use a seeding density of 3 x 10^5 to 5 x 10^5 viable cells/mL.
2. Incubate at 36-38°C in a humidified atmosphere of 8 % CO_2 in air on an orbital shaker platform rotating at 125 to 135 rpm. Loosen caps of flasks to allow for gas exchange.
3. Subculture once cell density reaches in excess of 1 x 10^6 cells/mL. Once consistent cell growth has been achieved, passage cells into a 50:50 ratio of CD CHO to original medium.
4. Repeat step 3 by increasing the ratio of CD CHO stepwise to original medium (75:25 followed by 90:10) until the cells are transferred into 100% CD CHO. Multiple passages at each step may be needed.
5. After several passages in 100% CD CHO, the viable cell count should reach 1 to 2 x 10^6 cells/mL with a viability exceeding 85% within 4-6 days of culture. At this stage the culture is considered to be adapted to CD CHO.

Cryopreservation

1. Prepare desired quantity of cells in either spinner or shaker culture, harvesting in mid-log phase of growth with viability >90%.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium (50% fresh CD CHO, 50% conditioned CD CHO + 7.5% DMSO) to give a final cell density of 0.5 to 1 x 10^7 cells/ mL.
3. Prepare the required volume of cryopreservation medium and store at 4°C until use; make cryopreservation medium on day of intended use.
4. Pellet the cells from culture medium at 100 x g for 5 to 10 minutes. Resuspend the pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications (i.e., 1.5 mL in a 2.0 mL cryovial).
6. Achieve cryopreservation in either an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Transfer frozen cells to liquid nitrogen, (vapor phase) storage at -125°C to -200°C is recommended.

Note: Viability and recovery of cryopreserved cells should be checked 24 hours after storage of vials in liquid nitrogen. ***See Recovery.***

Related Products

L-Glutamine, 200mM (100X), liquid (25030)

GLUTAMAX™ -I, 200mM (100X), liquid (35050)

CD DG44 Medium, (12610)

CHO CD EfficientFeed™ Kit, (A10241)

HT Supplement (100X), liquid (11067)

Water, Distilled, (15230)

Anti-Clumping Agent, (0010057AE)

GS Supplement 50X, (0010020DG)

Technical Support

For additional product and technical information, such as Material Safety Data Sheets (MSDS), Certificate of Analysis, etc, please visit our website at www.invitrogen.com. For further assistance, please email our Technical Support team at Techsupport@Invitrogen.com.

Note: A CD CHO Medium Master file has been submitted to the FDA. Permission to cross reference the Master file may be obtained by contacting Technical Support or your local Sales Representative.

Reference:

1. Gorfien, S.F., Dzimain, J.L., Tilkins, M.L., Godwin, G. P., & Fike, R. Recombinant Protein Production by CHO Cells Cultured in a Chemically Defined Medium. *Animal Cell Technology: Basic and applied Aspects*. Volume **9**, pg 247-252, Kluwer (Dordrecht), (1998).
2. Tilkins,M.L., Dzimain, J.L. Fike, R., Godwin, G.P., & Gorfien, S.F. Recombinant Protein Production by CHO Cells Cultured in Protein-Free and Serum-Free Media. Cell Culture Engineering V Meeting, San Diego, CA, January 28 - February 2,1996.
3. Gorfien, S.F., Dzimian, J., Tilkins, M.L., Godwin, G. & Fike, R. Recombinant Protein Production by CHO Cells Culture in a Chemically Defined Medium. The Japanese Association for Animal Cell Technology, 9th Annual Meeting, September 1-4, 1996, Yokohama, Japan.
4. Radominski, R., Hassett, R., Dadey, B., Fike, R., Cady, D. & Jayme, D. *Production-Scale Qualification of a Novel Cell Culture Medium Format. BioPharm*, Volume **14**, Number 7, (July 2001).
5. Radominski, R., Hassett, R., Dadey, B., Fike, R., Cady, D. & Jayme, D. *Advanced Granulation Technology (AGT) An alternate format for serum-free, chemically-defined and protein-free cell culture media*. Volume 36; 33-39, 2001.

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