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by life technologies

CD FortiCHO™ Medium

Description

CD FortiCHO™ Medium is animal origin-free and protein-free, containing no hydrolysates or components of unknown origin. CD FortiCHO™ Medium is specifically designed to offer high batch culture performance and yield with recombinant CHO cells [e.g., CHO-S® Cells (cGMP banked)] in a chemically defined environment. CD FortiCHO™ Medium is also provided as a component of the Freedom® CHO-S® Kit, an easy-to-use kit for the cloning and expression of recombinant proteins in CHO-S® cells. CD FortiCHO™ Medium provides consistency and reliability and reduces the need to screen for adventitious agents.

Product	Catalog no.	Amount	Storage	Shelf Life*
CD FortiCHO™ Medium	A11483-01	1000 mL	2°C to 8°C; Protect from light	12 months

* Shelf Life duration is determined from Date of Manufacture.

Product use

For Research Use Only. Not for use in diagnostic procedures.

Important information

- Hypoxanthine and thymidine free, for use in dihydrofolate reductase (DHFR) amplified systems.
- L-glutamine free, for use in Glutamine Synthetase systems.
- Formulated without phenol red to minimize estrogen-like effects.
- Glucose concentration is formulated to minimize potential lactic acid accumulation under typical culture conditions.
- Supports recombinant CHO cell growth and protein expression in suspension batch culture 2+ days longer than competitive alternatives.

Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Prepare medium

CD FortiCHO™ Medium requires aseptic supplementation with L-glutamine or GlutaMAX™-I prior to use.

1. Add GlutaMAX™-I or L-glutamine, 2–8 mM final concentration, to the medium before use.
2. Add 10 mL/L of HT Supplement for use in applications not requiring DHFR amplification.
3. Glucose supplementation may be required for terminal batch cultures and should be determined empirically.
4. Add Anti-Clumping Agent (1 mL/L) if required to the medium (after transfection) to reduce cell aggregation.

Note: consider reducing L-glutamine concentration for fed batch or perfusion protocols, or if the cell line in use is sensitive to ammonia. Addition of a surfactant such as Pluronic® F-68 is not required.

Culture Conditions

Media: CD FortiCHO™ Medium

Cell line: CHO cells

Culture type: Suspension

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 8% CO_2 in air

Culture vessels: shake flasks, spinner bottles (rpm may vary with shaker platform/impeller design and should be empirically determined for optimal cell growth), or bioreactor. Ensure proper gas exchange and minimize exposure of cultures to light.

Recovery

1. Rapidly thaw (<1 minute) frozen cells in a 37°C water bath.
2. Transfer the entire contents of the cryovial into a 125-mL shake flask containing 30 mL prewarmed complete CD FortiCHO™ Medium.
3. Incubate at 37°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 115–135 rpm.
4. Maintain cell density between 0.5–1 × 10^6 viable cells/mL for the first two passages following recovery; thereafter, return to your normal maintenance schedule.

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

Subculture cells

1. Determine viable cell density using a Countess® Automated Cell Counter (alternate automated or manual methods may be used).
2. Ensure that the cell density is ≥1 × 10^6 viable cells/mL, viability is ≥90%, and growth rate is in mid-logarithmic phase prior to subculturing.
3. Calculate the volume of cell culture and medium necessary to seed a flask at 2–3 × 10^5 viable cells/mL in a total volume of 30 mL fresh CD FortiCHO™ Medium per 125-mL shake flask. **Note:** If cell density does not reach 1 × 10^6 viable cells/mL within 5 days of recovery, centrifuge cells at 100 × *g* for 5 minutes and resuspend the cell pellet in 20–30 mL of fresh CD FortiCHO™ Medium.
4. Incubate at 37°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 115–135 rpm.
5. For optimal performance and cell growth dilute cells at a seeding density of 3 × 10^5 viable cells/mL every 3–4 days with fresh CD FortiCHO™ Medium.

Note: We recommend thawing a fresh low-passage vial of cells for use every 3 months or 30 passages.

Adaptation of CHO cells to CD FortiCHO™ Medium

We recommend adapting CHO cells to CD FortiCHO™ Medium using sequential adaptation. However, some CHO cell lines will adapt directly from other serum-free medium. It is critical that cell viability be ≥90% and the growth rate be in mid-logarithmic phase prior to initiating adaptation procedures.

Direct adaptation

1. For direct adaptation of CHO cells grown in other serum-free medium into CD FortiCHO™ Medium, dilute cells into 100% CD FortiCHO™ Medium using a seeding density of 3–4 × 10^5 viable cells/mL when subculturing. See **Subculture Cells**.

2. Continue to subculture cells at 3–4 × 10^5 viable cells/mL (every 3–4 days) until consistent growth is achieved. Once cell growth has been demonstrated the seeding density may be reduced to 2–3 × 10^5 viable cells/mL during the final stages of adaptation.
3. After several passages in CD FortiCHO™ Medium, the viable cell count should reach at least 2 × 10^6 cells/mL, with viability ≥85% within 3–4 days of seeding culture. At this stage, the culture is considered to be adapted to CD FortiCHO™ Medium.

Note: If suboptimal performance is achieved using the direct adaptation method, use the sequential adaptation method.

Sequential adaptation

1. During sequential adaptation of CHO cells grown in conventional 5–10% serum supplemented medium or other serum-free medium use a seeding density of 3–4 × 10^5 viable cells/mL.
2. Monitor cell growth using Countess® Automated Cell Counter until viable cell density reaches ≥1 × 10^6 cells/mL.
3. Dilute cells with a 25:75 ratio of complete CD FortiCHO™ Medium to the original media. We recommend maintaining backup cultures in the original ratio medium until success with the new ratio medium is achieved. Each passage dilute cells with stepwise increasing ratios of complete CD Hybridoma Medium to original medium with each subsequent passage (25:75, 50:50, 75:25, 90:10 followed by 100% CD FortiCHO™ Medium). Multiple passages at each step may be needed to achieve consistent growth.
4. After several passages in 100% CD FortiCHO™ Medium, the viable cell count should reach at least 2 × 10^6 cells/mL with a viability exceeding 85% within 3–4 days of seeding the culture. At this stage, the culture is considered to be adapted to CD FortiCHO™ Medium.

Cryopreservation

1. Prepare the desired quantity of cells, harvesting in mid-log phase of growth with viability >90%. Reserve the conditioned medium to prepare cryopreservation medium.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of >1 × 10^7 cells/mL.
3. Prepare the required volume of cryopreservation medium of 92.5% CD FortiCHO™ Medium (50:50 ratio of fresh-complete to conditioned media) +7.5% DMSO and store at 4°C until use.
   Important: Prepare cryopreservation medium on the day of use.
4. Harvest cells by centrifugation at 100 × *g* for 5–10 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.
6. Achieve cryopreservation in 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 –200°C to –125°C is recommended.
   Note: Check viability of cryopreserved cells 24 hours after storage of vials in liquid nitrogen. See **Recovery**.

Related products

Product	Catalog no.
L-Glutamine, 200 mM (100X), liquid	25030
GlutaMAX™-I, (100X), liquid	35050
HT Supplement, (100X), liquid	11067
Anti-Clumping Agent	0010057
Freedom® CHO-S® Kit	A13696-01
CHO-S® Cells (cGMP banked) and Media Kit	A11557-01
250X Cholesterol Lipid Concentrate	12531
FreeStyle™ MAX Reagent	16447
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227

Explanation of symbols and warnings

The symbols present on the product label are explained below:

				
Use By:	Manufacturer	Batch code	Keep away from light	Temperature Limitation
				
Catalog number	Consult instructions for use	Caution, consult accompanying documents	Sterilized using aseptic processing techniques	

Limited Use Label License: Internal Research and Bioproduction Use

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product (a) to perform internal research for the sole benefit of the purchaser; and (b) to culture cells for the purpose of producing a product wherein the product will be used for any or all of the following: (i) internal research use by the purchaser; (ii) resale for internal research use by third parties; (iii) performance of research conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties; (iv) resale for use as a human therapeutic agent or diagnostics product or component by third parties; (v) performance of manufacturing services conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties.
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