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CD OptiCHO™ Medium

Description

CD OptiCHO™ Medium has been developed for the growth of Chinese Hamster Ovary (CHO) cells and expression of recombinant proteins in suspension culture. CD OptiCHO™ is an animal origin-free (AOF), chemically defined medium that contains no proteins, hydrolysates, or components of unknown composition. CD OptiCHO™ is formulated without phenol red to minimize estrogen-like effects of phenol red.

Product	Catalog no.	Amount	Storage	Shelf life*
CD OptiCHO™ Medium (1X), liquid	12681-011 12681-029	1000 mL 6 × 1000 mL	2°C to 8°C; Protect from light 2°C to 8°C; Protect from light	18 months 18 months
CD OptiCHO™ AGT™ Medium**	A11222-04 A11222-05 A11222-01 A11222-03	1 L 1 × 10 L 1 × 100 L 10 kg	2°C to 8°C; Store dark and dry 2°C to 8°C; Store dark and dry 2°C to 8°C; Store dark and dry 2°C to 8°C; Store dark and dry	24 months 24 months 24 months 24 months

* Shelf life duration is determined from Date of Manufacture.
** AGT= Advanced Granulation Technology.

Product use

Caution: For manufacturing, processing, or repacking.

Safety information

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

Culture conditions

Media: CD OptiCHO™ Medium

Cell line: Chinese Hamster Ovary (CHO)

Culture type: Suspension

Culture vessels: Shake flasks, spinner bottles, or bioreactor.

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 8% CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Reconstitute CD OptiCHO™ AGT™

1. Measure room temperature (15–30°C) deionized or distilled water to 90% of final volume into an appropriately sized clean vessel.
2. Add CD OptiCHO™ AGT™ Medium at 19.3 g/L.
3. Mix with gentle stirring until medium dissolves completely. Do not heat.
4. Add deionized or distilled water to final volume.
5. Filter sterilize by 0.2 µm pore size membrane filtration.

   Note: Use low protein binding, low extractables filter.
6. Supplement as described in **Prepare medium** at time of use.

   Note: CD OptiCHO™ AGT™ Medium contains sodium bicarbonate. **Do not** add additional sodium bicarbonate. CD OptiCHO™ AGT™ Medium is auto pH and osmolality adjusted, no further adjustment required. For final lot pH and osmolality specifications, refer to Certificate of Analysis specification.

Prepare medium

CD OptiCHO™ and CD OptiCHO™ AGT™ Medium require supplementation with L-glutamine or GlutaMAX™-I prior to use.

1. Aseptically add L-glutamine or GlutaMAX™-I, 4–8 mM final concentration (20–40mL/L), to the medium before use.
2. CD OptiCHO™ 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 prior to use.
3. If cell clumping occurs, add 1 mL/L of Anti-Clumping Agent to medium. After any medium changes, subculture cells for a minimum of 3 passages before use in other applications.

   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.

Recovery

1. Rapidly thaw (<1 minute) frozen vial of cells in a 37°C water bath.
2. Transfer the entire contents of the cryovial into a 125-mL shake flask containing 28.5 mL of pre-warmed complete CD OptiCHO™ Medium.
3. Incubate at 37°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 125–135 rpm. Loosen flask caps to allow for gas exchange.
4. Subculture cells in mid-logarithmic phase 3–5 days post-thaw at a seeding density of 3×10^5 viable cells/mL. Subculture cells a minimum of 3 passages before use in other applications.

   Important: Do not centrifuge CHO cells upon thawing as they are extremely fragile upon recovery from cryopreservation.

Subculture cells

1. Determine viable cell density using a Countess® Automated Cell Counter or alternative automated or manual method.
2. Ensure that the cell density is 1×10^6 viable cells/mL, viability is at least 90%, and growth rate is in mid-logarithmic phase prior to sub culturing. If cell density does not reach 1×10^6 viable cells/mL within 5 days, centrifuge cells at $100 \times g$ for 5 minutes and resuspend cell pellet in 20–30 mL of fresh CD OptiCHO™ Medium.
3. 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 OptiCHO™ Medium.

   Note: It is recommended to thaw a fresh low-passage vial of cells at least every 3 months or 30 passages.

Adaptation of CHO Cells to CD OptiCHO™ Medium

We recommend adapting CHO cells to CD OptiCHO™ Medium using sequential adaptation. However, some CHO cell lines will adapt directly from other serum-free medium. To save time, you may choose to try both direct and sequential adaptation in parallel. It is critical that cell viability be at least 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 OptiCHO™ Medium, transfer cells into 100% CD OptiCHO™ Medium using a seeding density of 3×10^5–4×10^5 viable cells/mL when subculturing (see **Subculture Cells**).
2. Continue to subculture cells as necessary every 3–4 days at 3×10^5–4×10^5 viable cells/mL until consistent growth is achieved.
3. Once cell growth has been demonstrated the seeding density may be reduced to 2×10^5–3×10^5 viable cells/mL during the final stages of adaptation.
4. After several passages in CD OptiCHO™ Medium, the viable cell count should reach at least 2×10^6 cells/mL with viability exceeding 85% within 4–6 days of passage. At this stage, the culture is considered to be adapted to CD OptiCHO™ Medium.

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

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 complete CD OptiCHO™ Medium to the original media. During the adaptation procedure use a seeding density of 3×10^5–5×10^5 viable cells/mL.
2. Continue to subculture cells as necessary every 3–4 days when cell density reaches of 1×10^6 cells/mL.
3. Passage cells into incrementally greater proportions of CD OptiCHO™ Medium to original medium (75:25 followed by 90:10) once consistent cell growth has been achieved at each step until the cells are transferred into 100% complete CD OptiCHO™ Medium. Multiple passages at each step may be required.
4. After several passages in 100% complete CD OptiCHO™ Medium, the viable cell count should reach at least 2×10^6 cells/mL with viability exceeding 85% within 4–6 days of passage. At this stage the culture is considered to be adapted to CD OptiCHO™ Medium.

Cryopreservation

Prepare the desired quantity of cells harvesting in mid-log phase of growth with viability >90%. Save the conditioned medium to prepare cryopreservation medium.

1. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of $\geq 1 \times 10^7$ cells/mL.
2. Prepare the required volume of cryopreservation medium of 90% CD OptiCHO™ Medium (50:50 ratio of fresh to conditioned media) + 10% DMSO and store at 4°C until use.

   Note: Prepare cryopreservation medium on the day of intended use.
3. Harvest cells by centrifugation at 100 × *g* for 5–10 minutes.
4. Resuspend the cell 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.

Related products

Product	Catalog No.
L–Glutamine, 200mM (100X), liquid	25030
GlutaMAX™-I, 200mM (100X), liquid	35050
HT Supplement, (100X), liquid	11067
Anti-Clumping Agent	0010057
Water, Distilled	15230
Freedom® DG44 Kit	A13737
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227

Explanation of symbols and warnings

The symbols present on the product label are explained below:

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

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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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