gibco
life technologies

CHO-S-SFM II

Description

CHO-S-SFM II, is a complete, serum-free, low-protein (<100 µg/ml) medium optimized for the growth and maintenance of Chinese Hamster Ovary (CHO) cells in suspension culture and for the production of recombinant proteins expressed by CHO cells. This ready-to-use medium is suitable for the suspension culture of CHO and other cells in batch, continuous, and perfusion culture systems. Component deficient CHO-S-SFM II, is formulated without hypoxanthine and thymidine for use in dihydrofolate reductase (DHFR) amplified systems.

Product	Catalog no.	Amount	Storage	Shelf life*
CHO-S-SFM II	12052-114 12052-098	500 mL 1000 mL	2°C to 8°C; Protect from light 2°C to 8°C; Protect from light	12 months 12 months
CHO-S-SFM II, powder	22052-021 22052-047	10 × 1 L 1 × 50 L	2°C to 8°C; Store dark and dry	18 months
CHO-S-SFM II (without hypoxanthine and thymidine)	31033-020	500 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.

Product use (Cat. no. 12052 only)

Caution: For manufacturing, processing, or repacking.

Important information

CHO-S-SFM II is a complete medium, formulated with L-glutamine, and requires no further supplementation. Addition of hypoxanthine•Na at 1.66 mg/L and thymidine at 0.28 mg/L to component deficient CHO-S-SFM II, reconstitutes the original formulation of CHO-S-SFM II.

Safety information

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

Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV and HBsAg. Handle in accordance with established bio-safety practices.

Prepare media

Reconstitution

1. Add CHO-S-SFM II powder to 950 mL room temperature cell culture grade distilled water. Rinse the inside of package to remove all traces of powder.
2. Mix with gentle stirring until medium dissolves completely. **Do not heat**.
3. Add 2.45 g Sodium Bicarbonate ($NaHCO_3$) per liter of medium. Stir until dissolved.
4. Adjust medium to pH 8.0 with 1N NaOH while stirring. Slowly bring the pH to 7.0–7.1 with 1N HCl (Filtration will cause the pH to rise 0.1–0.3 units; final post-filtration pH should be 7.2–7.4).
5. Add deionized or distilled water to final volume of 1000 mL. Check pH and osmolality (Osmolality should be 320–345 mOsm/kg).
6. Filter sterilize through a 0.2-µm low extractables, low binding filter into clean, sterile containers. Store at 2°C to 8°C protected from light until use.

Culture conditions

Media: CHO-S-SFM II

Cell line: Chinese Hamster Ovary (CHO)

Culture type: Supension

Culture vessels: shake flask or spinner bottle or bioreactor system.

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.

Adapt CHO Cells to CHO-S-SFM II

Sequential adaptation of CHO cells from serum supplemented (or from the original CHO-S-SFM) may be required.

Note: It is critical that cell viability be at least 90% and cells be in the mid-logarithmic phase of growth prior to adaptation.

1. Subculture CHO cells grown in conventional medium with 5–10% serum or other serum-free medium into a 50:50 ratio of CHO-S-SFM II to the original media. During the adaptation procedure use a seeding density of 3×10^5–5×10^5 viable cells/mL.
2. Subculture cells when viable cell density reaches of 1×10^6 cells/mL.
3. Once consistent cell growth has been achieved, passage cells stepwise increasing the ratio of complete CHO-S-SFM II to original medium (75:25 followed by 90:10) until the cells are transferred into 100% CHO-S-SFM II. Multiple passages at each step may be required.
4. After several passages in 100% CHO-S-SFM II, the viable cell count should reach 1×10^6–3×10^6 cells/mL with viability exceeding 85% within 4–5 days of passage. At this stage the culture is considered to be adapted.

Note: It is not advisable to attempt to adapt cells already growing in serum-free formulations other than Gibco CHO-S-SFM to Gibco CHO-S-SFM II. Adaptation of cells grown in different serum-free media may be affected by selection of subpopulation(s) to specific components.

Cryopreservation

Prepare the desired quantity of cells in a tissue culture flask, harvesting in mid-log phase of growth with viability >90%. Reserve the conditioned medium to prepare cryopreservation medium.

1. Determine the viable cell density using a Countess® Automated Cell Counter, or alternative automated or manual method, and calculate the required volume of cryopreservation medium to give a final cell density of 0.5×10^7–1×10^7 cells/mL.
2. Prepare the required volume of cryopreservation medium of 92.5% CHO-S-SFM II (50:50 ratio of fresh to conditioned media) +7.5% DMSO and store at 4°C until use.

   Important: Prepare cryopreservation medium on the day of intended use.
3. Harvest cells by centrifugation at 100 × *g* for 5–10 minutes. Resuspend the cell pellet in the pre-determined volume of 4°C cryopreservation medium.
4. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications (i.e., 1.5 mL in a 2-mL cryovial).
5. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
6. 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.**

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 CHO-S-SFM II.
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. 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.

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

Related products

Product	Catalog no.
HT Supplement, (100X), liquid	11067
Anti-Clumping Agent	0010057
CHO CD EfficientFeed™ Kit	A10241
CHO CD EfficientFeed™ B AGT™	A12456
CD EfficientFeed™ C AGT™	A13275
CHO-S™ Cells (cGMP banked) and Media Kit	A11557-01
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	

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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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For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit **www.lifetechnologies.com/support**.
For further assistance, email **techsupport@lifetech.com**.

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