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CHO-S® Cells (cGMP Banked) and Media Kit

Description

CHO-S® Cells (cGMP Banked) and Media Kit have been developed for the growth of Chinese Hamster Ovary (CHO) cells and expression of recombinant proteins in suspension culture. CHO-S® cells have been adapted to CD CHO Medium for serum–free suspension growth, and subsequently banked and tested to meet cGMP quality standards. CD CHO Medium is an animal origin-free (AOF), chemically defined medium that contains no proteins, hydrolysates, or components of unknown composition. CD CHO Medium is formulated without L-glutamine for greater stability, and without phenol red to minimize potential for estrogen-like effects. CD CHO Medium is made without hypoxanthine and thymidine for use in dihydrofolate reductase (DHFR) amplified systems.

Product	Catalog no.	Amount	Storage	Shelf life*
CHO-S® Cells (cGMP Banked) and Media Kit Contains:	A11557-01	1 Kit		
CD CHO Medium	10743-029	1000 mL	2°C to 8°C; Protect from light	18 months
CHO-S® Cells (cGMP Banked)	A11364-01	1 vial**	–200°C to –125°C; Liquid Nitrogen	—
L-glutamine, 200mM	25030-081	100 mL	–20°C to –5°C; Protect from light	24 months
	25030-024†	100 mL	–20°C to –5°C; Protect from light	24 months

* Shelf Life duration is determined from Date of Manufacture.
** 1 vial contains ≥1 × 10^7cells/vial.
† For European Customers Only.

Product use

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

Important information

- CHO-S® cells have been produced, banked, and tested to meet current Good Manufacturing Practice regulations 21 CFR Parts 210, 211, 600, and 610.
- CHO-S® Cells: Stable when maintained at –200°C to –125°C.

Safety information

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

Prepare medium

CD CHO Medium requires supplementation with L-glutamine prior to use.

1. Aseptically add L-glutamine to 8 mM final concentration (40 mL/L), to the medium before use.
2. If cell clumping occurs, add 1 mL/L of Anti-Clumping Agent to medium. After any thaw or changes in media composition, 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 to reduce ammonia levels.

Note: Addition of a surfactant (e.g., Pluronic® F-68) is not required.

Culture Conditions

Media: Complete CD CHO Medium.

Cell Line: CHO-S® Cells (cGMP Banked).

Culture Type: Suspension

Culture Vessels: Shake flask or spinner bottle.

Temperature Range: 36°C to 38°C.

Incubator Atmosphere: Humidified atmosphere of 5–10% CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Recovery

1. Rapidly thaw (<2 minutes) 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 29 mL of prewarmed complete CD CHO Medium. If thawed properly, cell density should be ≥3 × 10^5 viable cells/mL, and viability should be ≥90%.
3. Incubate at 37°C in a humidified atmosphere of 5–10% CO_2 in air on an orbital shaker platform rotating at 125–135 rpm. Loosen flask caps (or use vented caps) to allow for gas exchange.
4. Subculture cells, 2–3 days post-thaw, when viable cell density reaches 1 × 10^6 cells/mL in mid-logarithmic phase of growth. Seed cultures at a 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-S® cells as they are extremely fragile upon recovery from cryopreservation.

Subculturing CHO-S® Cells in CD CHO Medium

Passage cells every 2–3 days into fresh medium. Repeat steps 1–4 as required to maintain or expand cultures.

1. Determine viable cell density and percent viability using a Countess® Automated Cell Counter (alternative automated or manual procedures may be used).
2. Determine the volume of cell culture suspension and fresh prewarmed complete CD CHO Medium needed to seed each new shake flask by dilution. Seed the culture at a density of 1–2 × 10^5 viable cells/mL.
3. Transfer the calculated volumes of prewarmed complete CD CHO Medium and cell suspension into a 125-mL shake flask. Loosen caps of flasks to allow for gas exchange.
4. Incubate at 37°C in a humidified atmosphere of 5–10% CO_2 in air, on an orbital shaker platform rotating at 125–135 rpm.

Note: CHO-S® viable cell densities can readily reach >6 × 10^6 cells/mL in CD CHO Medium, but clumping may occur at cell densities >2 × 10^6 cells/mL. It is recommended to thaw a fresh low-passage vial of cells every 3 months or 25 passages.

Transfection

CHO-S® cells can be transfected directly in CD CHO Medium using FreeStyle™ MAX transfection reagent after 5 passages without cell densities exceeding 2 × 10^6 viable cells/mL. Refer to the manual for transfection instructions. Other transfection reagents and methods can be used.

Note: Anti-Clumping Agent is incompatible with FreeStyle™ MAX and lipid-based transfections.

Scaling up CHO-S® Cells in CD CHO Medium

CHO-S® cultures can be scaled up in spinner bottles or stirred tank bioreactors using the following guidelines.

- Determine the optimum spinner or impeller speed for your bioreactor depending on culture requirements.
- Seeding density: We recommend an optimized seeding density of $1–2 \times 10^5$ viable cells/mL.
  Note: If the split ratio of cells to fresh media is <1:2, we recommend to spin down the cell suspension at 100 × *g* for 5–10 minutes, and resuspending the cell pellet in fresh complete CD CHO Medium prior to inoculating the spinner or bioreactor culture.

Cryopreservation

Prepare the desired quantity of cells in a tissue culture flask, harvesting in mid-log phase of growth when viable cell density reaches $>1 \times 10^6$ cells/mL with viability >90%.

1. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final viable cell density of $\geq 1 \times 10^7$ cells/mL.
2. Prepare the required volume of cryopreservation medium (90% fresh complete CD DG44 Medium, and 10% DMSO) and store at 4°C until use.
   Important: Prepare cryopreservation medium on the day of use.
3. Harvest cells by centrifugation at 100 × *g* for 5–10 minutes. Resuspend the 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**.

Related products

Product	Catalog no.
L–Glutamine-200mM (100X), Liquid	25030
Anti-Clumping Agent	0010057
FreeStyle™ MAX Reagent	16447
FreeStyle™ MAX CHO Expression System	K9000-20
EfficientFeed™ A+ AGT™ Supplement	A25023
EfficientFeed™ B+ AGT™ Supplement	A25030
EfficientFeed™ C+ AGT™ Supplement	A25031
CD CHO AGT™	12490
CD CHO Medium (1X), Liquid	10743
Water, Distilled	15230
Freedom™ CHO-S® Kit	A13696
Countess® Automated Cell Counter	C10227
Trypan Blue Stain	15250

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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