CHO DG44 Cells (cGMP Banked) and Media Kit and CD DG44 Medium

Catalog Numbers A11000-01, 12610-010

Pub. No. MAN0007386 **Rev.** 2.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Gibco™ CHO DG44 Cells (cGMP Banked) and Media Kit is optimized for the growth of dihydrofolate reductase deficient (DHFR-) Chinese Hamster Ovary (CHO) cells in suspension culture. The kit provides:

CD DG44 Medium— A chemically defined, protein-free, hydrolysate-free medium specifically designed to enable optimal growth of CHO DG44 cells in suspension culture. CD DG44 Medium contains hypoxanthine & thymidine (HT) for support of DHFR deficient cells, and is formulated without L-glutamine for greater stability, without phenol red to minimize potential for estrogen-like effects, and without Pluronic™ F-68. Media supplementation with L-glutamine and Pluronic™ F-68 (provided individually) is required for optimal growth of CHO DG44 cells in a protein-free, chemically defined environment.

CHO DG44 Cells (cGMP Banked)— Parental CHO DG44 cells have been produced, banked and tested to meet cGMP quality standards. They are pre-adapted to CD DG44 Medium and selected for superior cell growth.

Contents and storage

Table 1 CHO DG44 Cells (cGMP Banked) and Media Kit, Cat. No. A11000-01

Contents	Cat. No.	Amount	Storage	Shelf life[1]
CD DG44 Medium	12610-010	1000 mL	2°C to 8°C. Protect from light.	12 months
CHO DG44 Cells (cGMP Banked)	A10971-01	1 vial[2]	–200°C to –125°C. Liquid nitrogen.	—
Pluronic™ F-68	24040-032	100 mL	–20°C to –5°C. Protect from light.	24 months
L-Glutamine, 200 mM	A2916801	100 mL		

[1] Shelf-Life duration is determined from Date of Manufacture.
[2] 1 vial contains ≥ 1 × 10^7 cells/vial.

Table 2 CD DG44 Medium, Cat. No. 12610-010

Contents	Cat. No.	Amount	Storage	Shelf life[1]
CD DG44 Medium	12610-010	1000 mL	2°C to 8°C. Protect from light.	12 months

[1] Shelf-Life duration is determined from Date of Manufacture.

Important information

- The specified shaking speed (130–135 rpm) relates to a shaker-incubator with an orbital diameter (throw) of 25 mm. When using a shaker with a different orbital diameter, we recommended adjusting the shaking speed to match the relative centrifugal force (RCF) of 2.35–2.55.
  [RCF = 1.118×10^{-5} × ORBITAL RADIUS (mm) × SPEED^2 (rpm)]
- If cell clumping occurs, aseptically add 1 mL/L (1:1000) of Anti-Clumping Agent to medium. After thawing or any changes to the medium, subculture cells for a minimum of 3 passages before use in other applications.

Prepare complete CD DG44 Medium

CD DG44 Medium requires supplementation with L-glutamine and Pluronic™ F-68 prior to use.

1. Add 40 mL/L of freshly thawed 200 mM L-glutamine (8 mM final concentration) to the medium before use.
2. Add Pluronic™ F-68, 18 mL/L, to the medium before use.

IMPORTANT! Addition of this surfactant is required. Once supplemented, the complete CD DG44 Medium is stable for 1 month when properly stored at 2°C to 8°C protected from light.

Culture conditions

Medium: Complete CD DG44 Medium

Cell line: CHO DG44 Cells (cGMP Banked)

Culture type: Suspension

Culture vessels: Shake flask or spinner bottles

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.

Recovery

1. Rapidly thaw (<2 minutes) frozen vial of cells in a 37°C water bath.
2. Transfer the entire contents of the cryovial (≥1 × 10^7cells) into a sterile 125-mL shake flask containing 29 mL of pre-warmed complete CD DG44 Medium.

   If thawed appropriately, viability should be ≥90%, and viable cell density should be ≥3 × 10^5 cells/mL.
3. Incubate at 37°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 130–135 rpm. See "Important information".

   Loosen flask caps or use vented caps to allow for gas exchange.
4. Determine viable cell density and percent viability after 24–48 hours in culture using a Countess™ Automated Cell Counter (alternative automated or manual procedures may be used).
5. Subculture cells, 2–4 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 cells after thawing as they are extremely fragile upon recovery from cryopreservation.

Subculture CHO DG44 cells in CD DG44 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 complete CD DG44 Medium needed to seed each new shake flask by dilution. Seed the culture at a density of 3 × 10^5 viable cells/mL if subculturing every 2 days, or 2 × 10^5 viable cells/mL, if subculturing every 3 days.
3. Transfer the calculated volumes of pre-warmed complete CD DG44 Medium and cell suspension into a 125-mL shake flask.

   Ensure proper gas exchange.
4. Incubate at 37°C in a humidified atmosphere of 8% CO_2 in air, on an orbital shaker platform rotating at 130–135 rpm. See "Important information".

   Note: It is recommended to thaw a fresh low-passage vial of cells every 25 passages.

Cryopreservation

Prepare the desired quantity of cells by harvesting in mid-log phase of growth when viable cell density reaches >1 × 10^6 cells/mL with viability >90%.

1. Determine the viable cell density using a Countess™ Automated Cell Counter and calculate the required volume of cryopreservation medium to give a final viable cell density of ≥1 × 10^7 cells/mL.
2. Prepare the required volume of cryopreservation medium (90% fresh complete CD DG44 Medium + 10% DMSO) and store at 4°C until use.

   IMPORTANT! Prepare cryopreservation medium on the day of intended use.
3. Harvest cells by centrifugation at 300 × 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.
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).

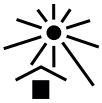
   Storage at –200°C to –125°C is recommended.

   Note: Check viability of cryopreserved cells 24 hours after storage of vials in liquid nitrogen. See "Recovery".

Related products

Product	Catalog No.
HT Supplement, (100X), liquid	11067
Anti-Clumping Agent	0010057
Pluronic™ F-68	24040
CHO CD EfficientFeed™ Kit	A10241
CHO CD EfficientFeed™ A AGT™ Kit	A14420
CHO CD EfficientFeed™ B AGT™ Supplement	A12456
CD EfficientFeed™ C AGT™ Supplement	A13275
CHO-S™ Cells (cGMP Banked) and Media Kit	A11000-01
Freedom™ DG44 Kit	A13737
Trypan Blue Stain	15250
Countess™ Automated Cell Counter	C10227

Explanation of symbols

				
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

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