gibco
by life technologies

FreeStyle™ CHO Expression Medium

Description

FreeStyle™ CHO Expression Medium is a serum-free, protein-free, chemically defined medium formulated with no components of animal, plant, synthetic or human origin and without phenol red to minimize potential for estrogen-like effects. FreeStyle™ CHO Expression Medium is optimized for the growth of CHO cells used in the FreeStyle™ Max CHO Expression System. The FreeStyle™ MAX CHO Expression System is a breakthrough technology for the rapid, high-yield production of post-translationally modified mammalian protein in less than one week. Transfection and expression experiments may be performed directly in FreeStyle™ CHO Expression Medium without the need to change media.

Product	Catalog no.	Amount	Storage	Shelf life*
FreeStyle™ CHO Expression Medium	12651-014 12651-022	1000 mL 6 × 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

FreeStyle™ CHO Expression Medium is not recommended for the culture of adherent CHO cell culture.

Safety information

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

Supplement medium

Supplement FreeStyle™ CHO Expression Medium with L-glutamine or GlutaMAX™-I prior to use.

1. Aseptically add L-glutamine or GlutaMAX™-I, 8 mM final concentration (40 mL/L), to the medium before use.
2. Once supplemented, store the complete FreeStyle™ CHO Expression Medium at 2°C to 8°C, protect from light.

Culture conditions

Media: FreeStyle™ CHO Expression Medium

Cell line: FreeStyle™ CHO-S cells

Culture type: Suspension

Culture vessels: Shake Flask, Spinner Bottle, 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.

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 tissue culture shake flask containing 30 mL of prewarmed complete FreeStyle™ CHO Expression Medium.
3. Incubate at 36°C to 38°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker set to 125–135 rpm.
4. Maintain a viable cell density between 0.5–1 × 10^6 viable cells/mL for the first two subcultures following recovery; thereafter, returning to the normal maintenance schedule.

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

Subculture cells

Subculture FreeStyle™ CHO-S cells directly into FreeStyle™ CHO Expression Medium.

1. Determine viable cell density using a Countess® Automated Cell Counter.
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 the cell density does not reach 1 × 10^6 viable cells/mL within 5 days of thawing, centrifuge cells at 100 × *g* for 5 minutes and resuspend the cell pellet in 20–30 mL of fresh FreeStyle™ CHO Expression 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 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% FreeStyle™ CHO Expression Medium (50:50 ratio of fresh to conditioned media) + 7.5% DMSO and store at 4°C until use.
   IMPORTANT! Prepare cryopreservation medium the day of use.
4. 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.
5. Immediately dispense aliquots of this cell suspension into cryovials according to the manufacturer's specifications (i.e., 1 mL in a 2-mL cryovial).
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; we recommend vapor phase storage at –200°C to –125°C.

Adapt cells to FreeStyle™ CHO Expression Medium

1. Ensure that the cell viability is at least 90%, and growth rate is in mid-logarithmic phase prior to adaptation. Subculture CHO cells grown in conventional medium with 5–10% serum or other serum-free medium into a 50:50 ratio of complete FreeStyle™ CHO Expression Medium to the original media. During the adaptation procedure seed at 6 × 10^5 viable cells/mL.
2. Monitor cell growth using Countess® Automated Cell Counter. When viable cell density reaches >1 × 10^6 viable cells /mL dilute the cells with an equal volume of complete FreeStyle™ CHO Expression Medium to a viable cell density of 3–5 × 10^5 viable cells/mL.
3. Repeat step 2. Continue to monitor and passage cells until consistent growth is achieved. At this point, the cells are considered to be adapted to FreeStyle™ CHO Expression Medium.

Transfect cells with FreeStyle™ Max Reagent

Recommendations

- Subculture CHO or FreeStyle™ CHO S cells in FreeStyle™ CHO Expression Medium, under standard culture conditions (see **Culture conditions**).
- Maintain cell densities within the range of 0.1–1.5 × 10^6 cells/mL of culture. Cell culture densities above 1.5 × 10^6 cells/mL will result in decreased transfection efficiency.
- One day prior to transfection, passage cells into complete FreeStyle™ CHO Expression Medium without antibiotics or Anti-Clumping Agent, both supplements may be added back post-transfection.

Transfect cells

1. 24 hrs before transfection, passage cells to 5–6 × 10^5 cells/mL seeding density. Place the flask(s) on an orbital shaker platform rotating at 120–135 rpm at 37°C, 8% CO_2.
2. On the day of transfection, confirm that the viable cell density is 1.2–1.5 × 10^6 cells/mL. Dilute cells to 1 × 10^6 cells/mL with fresh prewarmed FreeStyle™ CHO Expression Medium without antibiotics or Anti-Clumping Agent. To ensure optimal transfection, the viability of cells must be >95%. Add 30 mL cell suspension to each flask.
3. Mix FreeStyle™ MAX Reagent by gently inverting the tube several times. **Do not vortex**.
4. Dilute 37.5 µg of plasmid DNA into OptiPro™ SFM to a total volume of 0.6 mL and mix. In a separate tube, dilute 37.5 µL of FreeStyle™ MAX Reagent in OptiPro™ SFM to a total volume of 0.6 mL and mix gently by inversion (do not vortex). Immediately add diluted FreeStyle™ MAX Reagent to diluted DNA solution to obtain a total volume of 1.2 mL and mix by repeated gentle inversion.
5. Incubate the DNA-lipid mixture for 10 minutes at room temperature to allow complexes to form. Do not incubate for longer than 20 minutes.
6. Slowly add 1.2 mL of DNA-lipid mixture into the 125-mL flask containing cells while slowly swirling the flask.
7. Incubate transfected cell cultures at 37°C, 8% CO_2 on an orbital shaker platform rotating at 135 rpm. There is no need to change or supplement the culture medium during the first 6–7 days.
8. Protein expression can be detectable within 4 to 8 hours of transfection, with maximal protein yield usually between 1–7 days post-transfection, depending on the protein expressed.

Transfections may be scaled up or down using directly proportional volumes of cells and quantities of DNA.

Optimize protein expression

- When expressing a protein for the first time, perform a time course experiment between days 1–9 post-transfection to identify the peak of protein production, and to monitor cell viability.
- Test varying amounts of plasmid DNA and FreeStyle™ MAX Reagent. For 30 mL cultures, try a range between 24–45 µg plasmid DNA and 24–45 µL lipid.
- For secreted IgG protein production, we have observed peak yields at 5–7 days post-transfection.
- To assess transfection efficiency via a Green Fluorescent Protein (GFP) type fluorescent protein, we recommend monitoring the cultures starting at 24 hours post-transfection.
- For culture volumes above 30 mL, lowering the speed of the orbital shaker may be necessary if foam is generated. In 1 L cultures, we recommend 70–80 rpm.

Related products

Product	Catalog no.
FreeStyle™ MAX Reagent	16447
FreeStyle™ CHO-S Cells	R800-07
FreeStyle™ MAX CHO Expression System	K9000-20
L-Glutamine, 200 mM (100X), liquid	25030
GlutaMAX™-I, 200 mM (100X), liquid	35050
OptiPRO™ SFM (1X)	12309
FreeStyle™ 293-F Cells	R790-07
FreeStyle™ 293 Expression System	K9000-01
Penicillin-Streptomycin, (100X) liquid (5000 units)	15070
Anti-Clumping Agent	0010057
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 517: Internal Research and Bioproduction Use

Notice to Purchaser: The purchase of this product conveys to the purchaser the limited, non- transferable right to use the purchased amount of the product, under intellectual property rights that are owned and/or controlled by Life Technologies and relate specifically to the product, to perform (a) internal research for the sole benefit of the purchaser; and (b) research or manufacturing services conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties. The purchase of this product does not grant the purchaser any additional rights, including (without limitation) the right to transfer or resell the product in any form, the right to use the product as a therapeutic agent or diagnostics test component, or to use the product to perform tests other than what is indicated in this Limited Use Label License on a contract or fee per test basis for or on behalf of third parties. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

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**.

Important licensing information

These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

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**