

FreeStyle™ CHO Expression Medium

GIBCO FreeStyle CHO Expression Medium is developed for the growth of Chinese Hamster Ovary (CHO) cells and expression of recombinant proteins in suspension culture. The FreeStyle CHO Expression Medium is chemically defined and contains no proteins, hydrolysates, or components of unknown composition. This medium is animal origin free and all components have a known chemical structure.

Description	Cat. No.	Size
FreeStyle™ CHO Expression Medium	12651-014 12651-022	1000mL 6 x 1000 mL

Intended Use

FreeStyle CHO Expression medium is a serum-free, protein-free, chemically-defined medium for the growth of CHO cells and is intended for research use/further cell culture manufacturing. **CAUTION:** Not intended for human or animal diagnostic or therapeutic uses.

Features

- Chemically defined, containing no protein or polypeptide components of animal, plant or synthetic origin. There are also no undefined hydrolysates or lysates in the formulation.
- Formulated without L-glutamine.
- Superior growth of wild-type and recombinant CHO cells in suspension culture when compared to serum-free, undefined formulations.
- Formulated without phenol red to minimize potential for estrogen-like effects.

Precautions

- FreeStyle CHO Expression Medium requires supplementation with L-glutamine. Aseptically add 8 mM to the medium before use.

Storage

Store in the dark at 2 to 8°C

Shelf Life

12 months

Physical Conditions

Standard physical conditions for CHO cells grown in FreeStyle CHO Expression Medium are 37 ± 0.5°C in a humidified atmosphere of 8% CO₂ in air. Cultures may be grown in shake flasks (30 mL cell suspension in 125 mL shake flask) on an orbital shaker platform rotating at 125 to 135 rpm or in spinner flasks (rpm vary with impeller design). Loosen caps of flasks to permit gas exchange (vented caps can also be used). Avoid overexposure of cultures to light.

Recovery

- Rapidly thaw (≤ 1 minute) frozen vial in a 37°C water bath. Triturate and transfer the entire contents of the cryovial into a 125 mL shake flask containing 30 mL of pre-warmed FreeStyle CHO Expression Medium supplemented with 8mM L-glutamine. Incubate at 37°C in a humidified atmosphere of 8% CO₂ in air on an orbital shaker platform rotating at 125 to 135 rpm. Loosen caps of flasks to allow for aeration.
- Maintain the culture between 5 x 10⁵ and 1 x 10⁶ 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.

Cryopreservation

- Prepare desired quantity of cells in either spinner or shaker culture, harvesting in mid-log phase of growth with viability >90%.
- Determine the viable cell density and calculate the required volume of cryopreservation medium (50% fresh FreeStyle CHO Expression

Medium and 50% conditioned FreeStyle CHO Expression Medium + 7.5% DMSO) to give a final cell density of ≥1 x 10⁷ cells/mL.

- Prepare the required volume of cryopreservation medium and store at 4°C until use; make cryopreservation medium on day of intended use.
- Pellet the cells from culture medium at 100 x g for 5 to 10 minutes. Resuspend the pellet in the pre-determined volume of 4°C cryopreservation medium.
- Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications (i.e., 1.5 mL in a 2.0 mL cryovial).
- Achieve cryopreservation in either an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
- Transfer frozen cells to liquid nitrogen, (Vapor phase) storage at -125°C to -200°C is recommended.

Adaptation of CHO Cells to FreeStyle CHO Expression Medium

Sequential adaptation of CHO cells from serum supplemented or serum-free medium may be required. **It is critical that cell viability be at least 90% and cells be in the mid-logarithmic phase of growth prior to adaptation.** The recommended sequential adaptation procedure is as follows:

- Subculture the cell suspension grown in serum supplemented or serum-free medium into a 50:50 ratio (v/v) of FreeStyle CHO Expression Medium supplemented with 8mM L-glutamine and original medium at approximately 3-5 x 10⁵ cells/mL. Incubate the culture at 37±0.5°C in a humidified atmosphere of 8-10% CO₂ in air. Allow cell density to reach in excess of 1 x 10⁶ cells/mL.
- Subculture the above cell suspension in an equal volume of FreeStyle CHO Expression Medium to obtain a density of approximately 3-5 x 10⁵ cells/mL.
- Continue to subculture the cell suspension in FreeStyle CHO Expression Medium (using an inoculum of 3-5 x 10⁵ cells/mL) until the cell density reaches 1-2 x 10⁷/mL on day 4-6. At this point, the cells are considered to be adapted to FreeStyle CHO Expression Medium.

Transfection using FreeStyle Max Reagent

It is recommended to subculture CHO or FreeStyle CHO-S cells in Freestyle CHO Expression Medium, under standard physical conditions (see "Physical Conditions" section). Keep cell densities between 0.05 and 1.5x10⁶ cells/mL of culture. A cell density above 1.5x10⁶ cells/mL will result in a loss of transfection efficiency.

Use FreeStyle CHO Expression Medium supplemented with 8mM L-glutamine (Catalog no. 25030). **Do not** add antibiotics to media during transfection (this may cause cell death) or anti-clumping agent to the culture prior to transfection. Anti-clumping agent may be added post-transfection.

For other formats/volumes greater than 30mL, see "Scaling Up or Down Transfections" section. Refer to the FreeStyle CHO-S Cells manual for more details, available from our Web site (www.invitrogen.com) or Technical Service.

- Approximately 24 hrs before transfection, pass cells at 5-6 x 10⁵ cells/mL. Place the flask(s) on an orbital shaker platform rotating at 120-135 rpm at 37°C, 8% CO₂.

- On the day of transfection, the cell density should be about $1.2 - 1.5 \times 10^6$ cells/mL. Dilute the cells to 1×10^6 cells/mL with growth medium. To ensure optimal transfection, viability of cells must be > 95%. Add 30 mL of cells to each flask.

Transfecting Cells

- Gently invert the tube of FreeStyle MAX Reagent several times to mix. Do not vortex.
- 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 inverting the tube (do not vortex). Immediately add diluted FreeStyle MAX Reagent to diluted DNA solution to obtain a total volume of 1.2 mL and mix gently.
- Incubate the DNA-lipid mixture for 10 minutes at room temperature to allow complexes to form. Do not incubate for longer than 20 minutes.
- Slowly add 1.2 mL of DNA-lipid mixture into the 125 mL flask containing cells while slowly swirling the flask.
- Incubate transfected cell cultures at 37°C, 8% CO₂ on an orbital shaker platform rotating at 135 rpm. There is no need to change or supplement the culture medium during the first 6 to 7 days.
- Protein expression can be detectable within 4 to 8 hours of transfection, with maximal protein yield usually between 1 and 7 days post-transfection, depending on the protein expressed.

Optimizing Protein Expression

- When expressing a protein for the first time, perform a time course experiment between days 1 and 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 to 7 days post-transfection.
- To assess transfection efficiency via a GFP (Green Fluorescent Protein) type fluorescent protein, we recommend monitoring the cultures starting at 24 hours post-transfection.

Scaling Up or Down Transfections

To transfect cells in different culture volumes, vary the amounts of FreeStyle MAX Reagent, DNA, cells and medium in proportion to the culture volume, as indicated in the following table:

Cell Culture		Dilution	DNA		FreeStyle™ MAX Reagent	
Volume	Flask		Starting Point	Optimization Range	Starting Point	Optimization Range
30 mL	125 mL	2 x 0.6 mL	37.5 µg	24-45 µg	37.5 µl	24-45 µl
250 mL	1 liter	2 x 5 mL	312.5 µg	200-375 µg	312.5 µl	200-375 µl
1 liter	3 liter	2 x 20 mL	1.25 mg	0.8-1.5 mg	1.25 mL	0.8-1.5 mL

For culture volumes **above 30 mL**, lowering the speed of the orbital shaker may be necessary if foam is generated. In 1L cultures, we recommend 70-80 rpm.

Quality Control

FreeStyle CHO Expression Medium is performance tested using CHO-S cells in a dynamic culture system. Additional standard evaluations are endotoxin, pH, osmolality and tests for the absence of bacterial and fungal contaminants.

Related Products

FreeStyle™ MAX Reagent (16447-100)

FreeStyle™ CHO-S cells in FreeStyle™ CHO Expression Medium (R800-07)

FreeStyle™ MAX CHO Expression System (K9000-20)

L-Glutamine-200 mM, 100X, (25030-081)

OptiPro™ SFM 1X, (12309-050, 100mL)

Anti-Clumping Agent (01-0057AE)

Trypan Blue Stain (15250-061)

Contacts

For further information on this or other GIBCO® products, contact Technical Services at the following:

United States TECH-LINESM : 1 800 955 6288

Canada TECH-LINE: 1 800 757 8257

Outside the U.S. and Canada, refer to the GIBCO products catalogue for the TECH-LINE in your region.

You may also contact your Invitrogen Sales Representative or our World Wide Web site at www.invitrogen.com.

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References

- Gorfien, S.F., Dzimain, J.L., Tilkins, M.L., Godwin, G. P., & Fike, R. Recombinant Protein Production by CHO Cells Cultured in a Chemically Defined Medium. *Animal Cell Technology: Basic and Applied Aspects*. Volume 9, pg 247-252, Kluwer (Dordrecht), (1998).
- Tilkins, M.L., Dzimain, J.L. Fike, R.M, Godwin, G.P., & Gorfien, S.F. Recombinant Protein Production by CHO Cells Cultured in Protein-Free and Serum-Free Media. Cell Culture Engineering V Meeting, San Diego, CA, January 28 - February 2, 1996.
- Gorfien, S.F., Dzimian, J.L., Tilkins, M.L., Godwin, G.P. & Fike, R.M. Recombinant Protein Production by CHO Cells Culture in a Chemically Defined Medium. The Japanese Association for Animal Cell Technology, 9th Annual Meeting, September 1-4, 1996, Yokohama, Japan.

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