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FreeStyle™ CHO-S™ cells

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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Revision	Date	Description
B.0	16 February 2020	Converted to CCMS. Rebranded. Updated contents to state liquid nitrogen vapor phase. Added the Important guidelines for thawing and storing cells topic.
A.0	21 April 2015	Remove RUO statement, add LULL 561, update trademark language and branding. Discontinue printed p/n 250921.

Contents

FreeStyle™ CHO-S™ cells

Contents and storage

Shipping and storage

This manual is shipped with FreeStyle™ CHO-S™ Cells. FreeStyle™ CHO-S™ Cells are shipped on dry ice. Upon receipt, store in **liquid nitrogen vapor-phase**.

Contents

Storage conditions: Liquid nitrogen vapor-phase

Amount supplied: One vial containing 1×10^7 cells.

Composition: 1 mL of cells in 90% FreeStyle™ CHO™ Expression Medium and 10% DMSO.

CAUTION! Handle as potentially biohazardous material under at least Biosafety Level 1 containment.

WARNING! GENERAL CHEMICAL HANDLING. For every chemical, 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 **www.lifetechnologies.com/support**.

This product contains Dimethyl Sulfoxide (DMSO); components of the product may be absorbed into the body through the skin.

Important guidelines for thawing and storing cells

- Upon receipt, immediately thaw cells or place into vapor-phase liquid nitrogen storage until ready to use. **Do not store the cells at –80°C**.
- Avoid short-term extreme temperature changes. When storing cells in liquid nitrogen after shipping on dry ice, allow the cells to remain in liquid nitrogen for 3-4 days before thawing.
- Prior to starting experiments, ensure you have established cells and have frozen stocks on hand. Upon receipt, grow and freeze multiple vials of cells to ensure that you have an adequate supply of early-passage cells.

Introduction

Overview

Introduction

FreeStyle™ CHO-S™ Cells are derived from the CHO-S™ cell line (see "Parental cell line" on page 6), and are adapted to suspension culture in FreeStyle™ CHO™ Expression Medium. FreeStyle™ CHO-S™ Cells are designed for use with the FreeStyle™ MAX CHO™ Expression System to facilitate high level production of a protein of interest. Frozen cells are supplied in and may be thawed directly into FreeStyle™ CHO™ Expression Medium (see "Thaw and establish cells" on page 10).

Parental cell line

Chinese Hamster Ovary (CHO™) cells are among the most commonly used cell lines for transfection, expression and large-scale production of recombinant proteins. The CHO-S™ cell line is a stable aneuploid cell line established from the ovary of an adult Chinese hamster (Puck, 1958). The cell line has been distinguished as a separate sub-clone from the common CHO™ K1 cell line, and its history and stability have been extensively described (D'Anna, 1996; D'Anna et al., 1997; Deaven & Petersen, 1973).

FreeStyle™ CHO-S™ cells

- Prepared from low passage Master Cell Bank cultures derived from parental CHO-S™ cells that were re-cloned by limiting dilution, and selected for their superior serum-free cell growth and transfection efficiencies. The clonally derived cultures are maintained in serum-free conditions for only 20–25 total passages.
- CHO-S™ Master Cell Bank cultures have been tested by an independent service and found to be negative for HBV, HCV, HTLV-I & -II, and HIV-1 & -2.
- Adapted to serum-free suspension growth in FreeStyle™ CHO™ Expression Medium, a chemically defined, serum-free, and protein-free medium (Gorfien, 1998). See "FreeStyle™ CHO™ expression medium" on page 6 for more information.

  Note: Cells also grow well in traditional media supplemented with serum.
- Suspension cultures may be transfected in FreeStyle™ CHO™ Expression Medium without the need to change media.
- Demonstrates high transfection efficiency with FreeStyle™ MAX Reagent. See "FreeStyle™ MAX reagent" on page 7 for more information.
- Permits transfection of cells at large volumes, from shake flasks to bioreactors.

FreeStyle™ CHO™ expression medium

FreeStyle™ CHO™ Expression Medium is a defined, serum-free medium specifically developed for the high-density, suspension culture and transfection of CHO™ cells. The medium contains **no** human or animal origin components. For more information, visit **www.lifetechnologies.com** or call Technical Support (see).

Important

The FreeStyle™ CHO™ Expression Medium should be supplemented with L-glutamine to a final concentration of 8 mM before use.

Growth of FreeStyle™ CHO-S™ cells in the medium

Typically, FreeStyle™ CHO-S™ cells cultured in FreeStyle™ CHO™ Expression Medium demonstrate the following:

- Doubling time in the range of 16–22 hours (doubling time can exceed 22 hours during the first few passages after the cells have been thawed.)
- Cell densities of up to 7×10^6 cells/mL in shaker or spinner culture
- Cell densities of up to 4×10^6 cells/mL in bioreactor culture

For general cell culture, keep FreeStyle™ CHO-S™ cells between 5×10^4 and 1.5×10^6 cells/mL before transfection (or between 0.5×10^6–2×10^6 cells/mL if passaging daily). A cell density that is too high will result in decreased transfection efficiency.

If large numbers of cells are needed, you can seed cultures at 0.5×10^6 cells/mL and use cells **as soon as** they reach a density of 5×10^6 cells/mL (3–4 days). Do not let cultures that have reached a cell density of 5×10^6 cells/mL grow any further, as this will result in decreased transfection efficiency.

Note: Individual culturing and passaging techniques coupled with cellular heterogeneity inherent within the FreeStyle™ CHO-S™ cell population may result in experimental variability.

FreeStyle™ MAX reagent

FreeStyle™ MAX Reagent is a proprietary, animal origin-free formulation for the highly efficient transfection of plasmid DNA into eukaryotic cells. FreeStyle™ MAX Reagent is specifically formulated to achieve the **highest expression levels** and **lowest cytotoxicity** in suspension FreeStyle™ CHO-S™ Cells and FreeStyle™ 293-F Cells.

For more information, visit **www.lifetechnologies.com** or call Technical Support (see).

FreeStyle™ MAX CHO™ expression system

The FreeStyle™ CHO-S™ Cells are part of the FreeStyle™ MAX CHO™ Expression System. The FreeStyle™ MAX CHO™ Expression System is the first commercially available optimized system for transient transfection and protein production in CHO™ cells. The FreeStyle™ MAX CHO™ Expression System provides the following advantages:

- Uses Chinese Hamster Ovary cells, the most widely used cell line for expression of recombinant protein in mammalian cells. This will streamline the process for clinical applications.
- The FreeStyle™ MAX Reagent offers high recombinant protein yield with low cytotoxicity
- Provides a rapid transient transfection protocol for expression of your target protein.
- Uses suspension culture to easily scale up to large amounts of culture.

- All reagents are completely animal-origin free, including the defined, serum-free medium, which may be imperative for regulatory requirements
- CHO™ cells are known to provide stable and accurate glycosylation (Sheeley et al., 1997; Werner et al., 1998), and are more likely to yield accurate glycoproteins.

For more information, visit **www.lifetechnologies.com** or call Technical Support (see).

Methods

Important guidelines

General cell handling

Follow the general guidelines below to grow and maintain FreeStyle™ CHO-S™ cells.

- **All solutions and equipment that come in contact with the cells must be sterile.** Always use proper sterile technique and work in a laminar flow hood.
- Before starting experiments, be sure to have cells established (at least 5 passages) and also have some frozen stocks on hand. We recommend using early-passage cells for your experiments. Upon receipt of the cells, grow and freeze multiple vials of the FreeStyle™ CHO-S™ cell line to ensure that you have an adequate supply of early-passage cells.
- For general maintenance of cells, pass FreeStyle™ CHO-S™ cells when they reach a density of approximately 1×10^6–1.5×10^6 viable cells/mL (generally every 48–72 hours).
- Alternatively, cells can be passed every day. If passing cells every day, pass FreeStyle™ CHO-S™ cells when they reach a density of approximately 2×10^6 viable cells/mL.
- If large numbers of cells are needed, you can seed cultures at 0.5×10^6 cells/mL and use cells **as soon as** they reach a density of 5×10^6 cells/mL (3–4 days). Do not let cultures that have reached a cell density of 5×10^6 cells/mL grow any further, as this will result in a decrease of transfection efficiency.
- Use trypan blue exclusion to determine cell viability (see “Determine cell density and viability” on page 10). Log phase cultures should be >95% viable.
- When thawing or subculturing cells, transfer cells into pre-warmed medium.

CAUTION! As with other human cell lines, when working with FreeStyle™ CHO-S™ cells, handle as potentially biohazardous material under at least Biosafety Level 1 containment.

Media preparation

For suspension growth and transfection applications for FreeStyle™ CHO-S™ cells, use:

- FreeStyle™ CHO™ Expression Medium. This medium should be supplemented with L-glutamine to a final concentration of 8 mM before use (e.g., 40 mL of 200 mM stock to one liter of medium).
- 5 mL/L of Penicillin/Streptomycin (0.5X Pen Strep) may be used when required

See “Accessory products” on page 22 for ordering information.

Important

FreeStyle™ CHO™ Expression Medium is extremely sensitive to light. For optimal results, use and store media protected from light.

Determine cell density and viability

Use the following procedure to determine viable and total cell counts.

1. Transfer™ a small aliquot of the cell suspension to a microcentrifuge tube.
2. Determine viability and the amount of cell clumping using the trypan blue dye exclusion method (see "Accessory products" on page 22 for ordering information).
3. Determine cell density electronically using a Coulter Counter or manually using a hemacytometer.

Thaw and establish cells

Introduction

Follow the provided protocol to thaw FreeStyle™ CHO-S™ cells to initiate cell culture. The FreeStyle™ CHO-S™ cell line is supplied in a vial containing 1 mL of cells at 1 × 10^7 viable cells/mL in 90% FreeStyle™ CHO™ Expression Medium and 10% DMSO. Thaw FreeStyle™ CHO-S™ cells directly into the FreeStyle™ CHO™ Expression Medium.

Materials needed

You will need to have the following reagents on hand before beginning:

- FreeStyle™ CHO-S™ cells (supplied; store frozen cells in liquid nitrogen until ready to use)
- FreeStyle™ CHO™ Expression Medium (pre-warm at 37°C before use). Make sure that 8 mM L-Glutamine has been added. See "Accessory products" on page 22 for ordering information.

  Note: Do not add antibiotics to media at this point as this may impact cell growth.
- 125-mL polycarbonate, disposable, sterile Erlenmeyer flask with vented cap (available from VWR™, Radnor PA, Cat. no. 30180-036)
- Orbital shaker in 37°C incubator with a humidified atmosphere of 8% CO_2
- Reagents to determine viable and total cell counts (see "Determine cell density and viability" on page 10)

Thaw procedure

Store frozen cells in liquid nitrogen until ready to use. To thaw and establish cells:

1. Remove the cryovial of cells from the liquid nitrogen and thaw quickly in a 37°C water bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol. Gently break up clumps and cell pellet if present and transfer the entire contents of the cryovial into a 125-mL polycarbonate, disposable, sterile Erlenmeyer shaker flask containing 30 mL of pre-warmed FreeStyle™ CHO™ Expression Medium supplemented with 8 mM L-Glutamine.
3. Incubate cells in a 37°C incubator containing a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 125 rpm.
4. The next day, determine viable and total cell counts (see protocol in "Determine cell density and viability" on page 10). Generally, viability is >70%; a bit lower is no reason for concern, but if viability is less than 60% thaw a new batch of cells.
5. Subculture the FreeStyle™ CHO-S™ cells 24–48 hours after thawing by seeding cells at 0.3 × 10^6 viable cells/mL in pre-warmed FreeStyle™ CHO™ Expression Medium supplemented with 8 mM L-Glutamine. We generally use 125- or 250-mL polycarbonate, disposable, sterile, Erlenmeyer flasks containing 40 mL or 80 mL total working volume of cell suspension, respectively.

IMPORTANT! Subculture cells a minimum of five passages before use in transfection experiments to allow opportunity for recovery from thawing. To subculture cells, see the procedure in "Subculture cells" on page 11.

Subculture cells

Passaging cells every 48–72 hours

Subculture cells when the density is approximately 1 × 10^6–1.5 × 10^6 viable cells/mL, typically every 48–72 hours. When maintaining FreeStyle™ CHO-S™ cells, we generally use a 125- or 250-mL polycarbonate, disposable, sterile Erlenmeyer flask with vented cap containing 40 mL or 80 mL total working volume of cell suspension, respectively.

1. Determine viable and total cell counts (see protocol in "Determine cell density and viability" on page 10).
2. Using the cell density determined in Step 1 on page 11, calculate the split ratio needed to seed the new shaker flask at
3. 0.5 × 10^5–2 × 10^5 viable cells/mL.
4. Dilute the cells in fresh, pre-warmed FreeStyle™ CHO™ Expression Medium supplemented with 8 mM L-Glutamine to give a final cell density of 0.5 × 10^5–2 × 10^5 viable cells/mL in the desired final volume.

5. Incubate flasks in a 37°C incubator containing a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 120–135 rpm.

6. Repeat Steps 1 on page 11–4 on page 12 as necessary to maintain or expand cells.

Passaging cells every 24 hours

Alternatively, cells may be passaged every 24 hours. Split cells to approximately 0.5 × 10^6 viable cells/mL as described above. The next day, cell density should be 1.3 × 10^6–1.4 × 10^6 viable cells/mL. Split cells every 24 hours.

If expansion is needed, cells can be split at 0.8 × 10^6–1.0 × 10^6 viable cells/mL. Next day, cell density should be 2.0 × 10^6 viable cells/mL. Split cells every 24 hours.

Passaging large numbers of cells

If large numbers of cells are needed, you can seed cultures at 0.5 × 10^6 cells/mL and use cells **as soon as** they reach a density of 5 × 10^6 cells/mL (3–4 days). Do not let cultures that have reached a cell density of 5 × 10^6 cells/mL grow any further, as this will result in a decrease of transfection efficiency.

Shake flasks

The cells can be grown in many different culture volumes. We generally use the following polycarbonate, disposable, sterile Erlenmeyer flask with vented cap (other flasks with the same characteristics may be used): For culture volumes **above 40 mL**, lower the speed of the orbital shaker if foam is generated. In 1 L cultures, we recommend 70–80 rpm.

Flask volume	Culture volume	Manufacturer	Catalog no.
125-mL	25–40 mL	VWR™, Radnor, PA	30180-036
250-mL	50–80 mL	VWR™, Radnor, PA	30180-044
500-mL	100–150 mL	VWR™, Radnor, PA	30180-052
1-L	200–300 mL	VWR™, Radnor, PA	82013-164
3-L	600–1000 mL	Corning™, Acton, MA	431252

Note: Glass flasks without baffles may be used, but thorough cleaning after each use is essential to avoid potential toxicity which is more problematic in serum-free cultures.

Other cell culture systems

It is possible to scale up the FreeStyle™ CHO-S™ cultures in spinner flasks, stirred bioreactors, or wave bags. The appropriate spinner, impeller, or shaking speed and seeding density should be determined and optimized for each system. We recommend seeding cells at 0.2×10^6 viable cells/mL.

Note: Monitor cell viability, foaming and the degree of cell clumping. If foam is generated, lower agitation speed. Note that extensive cell clumping may reduce transfection efficiency.

Cryopreservation

Introduction

You may freeze FreeStyle™ CHO-S™ cells directly in FreeStyle™ CHO™ Expression Medium with 10% DMSO. When freezing the FreeStyle™ CHO-S™ cell line, we recommend the following:

- Freeze cells at a density of $\geq 1 \times 10^7$ viable cells/mL.
- Use a freezing medium composed of 90% fresh growth medium and 10% DMSO.

Guidelines to prepare freezing medium and to freeze cells are provided in this section.

Prepare freezing medium

Prepare freezing medium immediately before use.

1. In a sterile, conical centrifuge tube, mix together the following reagents for every 1 mL of freezing medium needed:

FreeStyle™ CHO™ Expression Medium	0.9 mL
DMSO	0.1 mL

2. Filter-sterilize the freezing medium and place the tube on ice until use. Discard any remaining freezing medium after use.

Freeze cells

Before starting, label cryovials and prepare freezing medium. Keep the freezing medium on ice.

1. Grow the desired quantity of FreeStyle™ CHO-S™ cells in shaker flasks, harvesting when the cell density reaches 1×10^6 viable cells/mL. Transfer™ cells to a sterile, conical centrifuge tube.

2. Determine the viable and total cell counts (see protocol in “Determine cell density and viability” on page 10) and calculate the volume of freezing medium required to yield a final cell density of 1×10^7 viable cells/mL.

3. Centrifuge cells at $100 \times g$ for 5 minutes at room temperature and carefully aspirate the medium.

4. Resuspend the cells in the pre-determined volume of chilled freezing medium.

5. Place cryovials in a microcentrifuge rack and aliquot 1 mL of the cell suspension into each cryovial.

6. Freeze cells in an automated or manual, controlled-rate freezing apparatus following standard procedures. For ideal cryopreservation, the freezing rate should be a decrease of 1°C per minute.

7. Transfer™ frozen vials to liquid nitrogen vapor-phase for long-term storage.

Note: You may check the viability and recovery of frozen cells 24 hours after storing cryovials in liquid nitrogen vapor-phase by following the procedure outlined in "Thaw and establish cells" on page 10.

Transfect cells

Introduction

To transfect suspension FreeStyle™ CHO-S™ cells, use the cationic lipid-based transfection reagent, FreeStyle™ MAX Reagent. Unlike some other serum-free media formulations, FreeStyle™ CHO™ Expression Medium does not inhibit cationic lipid-mediated transfection. FreeStyle™ CHO™ Expression Medium is specifically formulated to allow high transfection efficiency of suspension FreeStyle™ CHO-S™ cells **without** the need to change or add media. Transient transfection experiments may be performed in a large volume, allowing larger-scale protein production.

FreeStyle™ CHO™ Expression Medium and FreeStyle™ MAX Reagent is available separately from Thermo Fisher Scientific (see "Accessory products" on page 22 for ordering information). For more information, see the FreeStyle™ MAX CHO™ Expression System, available from our website (**www.lifetechnologies.com**) or call Technical Support (see).

FreeStyle™ MAX reagent

FreeStyle™ MAX Reagent is a proprietary formulation suitable for transfection of DNA into eukaryotic cells grown in suspension. In the FreeStyle™ MAX CHO™ Expression System, use of FreeStyle™ MAX Reagent to transfect FreeStyle™ CHO-S™ cells provides the following advantages:

- FreeStyle™ MAX Reagent demonstrates high transfection efficiency with minimal cytotoxicity in suspension FreeStyle™ CHO-S™ cells (cultured in FreeStyle™ CHO™ Expression Medium)
- DNA-FreeStyle™ MAX Reagent complexes can be added directly to cells in culture medium
- It is not necessary to remove complexes or change or add medium following transfection

Plasmid preparation

Plasmid DNA for transfection into eukaryotic cells must be clean, sterile and free from phenol and sodium chloride. Contaminants may kill the cells, and salt will interfere with complexing, decreasing transfection efficiency. We recommend isolating DNA using the PureLink™ HiPure Plasmid Kits, which are validated for use with the FreeStyle™ MAX CHO™ Expression System (see "Accessory products" on page 22).

Note: Make sure your DNA preparation is sterile. We recommend performing filtration before use through a 0.22 µm filter.

Materials needed

- Suspension FreeStyle™ CHO-S™ cells cultured in FreeStyle™ CHO™ Expression Medium

  Note: Calculate the number of cells that you will need for your transfection experiment and expand cells accordingly. Make sure that the cells are healthy and ≥95% viable before proceeding to transfection.
- Purified plasmid DNA of interest (1 mg/mL)
- FreeStyle™ MAX Reagent (store at 4°C until use)
- OptiPRO™ SFM™ (pre-warmed to room temperature)
- FreeStyle™ CHO™ Expression Medium, supplemented with L-glutamine to a final concentration of 8 mM (pre-warmed to 37°C)

  Note: Do not add more than 5 mL/L of Penicillin/Streptomycin (0.5X Pen Strep) to media during transfection as this may decrease transfection activity
- 125-mL polycarbonate, disposable, sterile Erlenmeyer flasks
- Orbital shaker in 37°C incubator with a humidified atmosphere of 8% CO_2
- Reagents to determine viable and total cell counts

FreeStyle™ CHO™ Expression Medium, OptiPRO™ SFM™ and FreeStyle™ MAX Reagent are available separately from Thermo Fisher Scientific (see "Accessory products" on page 22 for ordering information).

Optimal conditions for 30 mL transfection

To transfect suspension FreeStyle™ CHO-S™ cells in a 30-mL volume, we recommend using the following optimized conditions:

- **Final transfection volume:** 30 mL
- **Number of cells to transfect:** 3×10^7 cells (final cell density of 1×10^6 cells/mL)
- **Amount of plasmid DNA:** 37.5 µg (starting point; can vary from 30–45 µg)
- **FreeStyle™ MAX Reagent:** 37.5 µL (starting point; can vary from 30–45 µL)

Transfection procedure

Follow the procedure below to transfect suspension FreeStyle™ CHO-S™ cells in a **30-mL volume**. Remember that you may keep the cells in FreeStyle™ CHO™ Expression Medium during transfection. We recommend including a positive control (pCMV SPORT-βgal) and a negative control (no DNA, no FreeStyle™ MAX Reagent) in your experiment to help you evaluate your results.

1. Approximately 24 hours before transfection, pass FreeStyle™ CHO-S™ cells at 5 × 10^5–6 × 10^5 cells/mL. 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, the cell density should be about 1.2 × 10^6–1.5 × 10^6/mL. Dilute the cells to 1 × 10^6 cells/mL. To ensure high transfection results, viability of cells must be ≥95%. Add 30 mL of cells into each 125-mL shake flask.

3. Gently invert the tube of FreeStyle™ MAX Transfection Reagent several times to mix. 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 Transfection 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 Transfection Reagent to diluted DNA solution to obtain a total volume of 1.2 mL and mix gently.

5. Incubate the DNA-FreeStyle™ MAX mix 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-FreeStyle™ MAX Reagent complex 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 may be detectable within 4–8 hours of transfection, with maximal protein yield usually between 1–7 days post-transfection.

Optimize protein expression

- When expressing a protein for the first time, perform a time course experiment between days 1–7 post-transfection to identify the peak of protein production, and to monitor cell viability.
- We have observed peak yields for IgG protein production at 5–7 days post-transfection.
- Test varying amounts of plasmid DNA and FreeStyle™ MAX Reagent. For 30 mL cultures, try between 30–45 µg DNA and 30–45 µL FreeStyle™ MAX Reagent.
- To assess transfection efficiency via expression of a GFP–type fluorescent protein, we recommend monitoring the cultures starting at 24 hours post-transfection.
- In some cases, transfection efficiency may decrease during the course of the experiment, although protein production is still increasing. Never evaluate results by transfection efficiency only; always measure the protein production.

For optimizing protein expression while scaling up culture volumes, see “Scale up transfections” on page 17.

Note: Cells that are transfected with high efficiency grow slower than untransfected cells. This is a good thing.

Scale up transfections

If you transfect suspension FreeStyle™ CHO-S™ cells in a larger volume, consider the following points:

- Scale up the volume of each reagent in proportion to the culture volume.
- For culture volumes **above 30 mL**, lower the speed of the orbital shaker if foam is generated. In 1 L cultures, we recommend 70–80 rpm.
- The transfection conditions may vary depending on the type of culture vessel used and the growth conditions of your cells; therefore, you may want to perform pilot studies to optimize your transfection conditions.
- Often, the conditions for transfection of large culture volumes need to be adjusted to the higher side of the range, while those for small culture volumes may tend towards the lower side of the range.

Troubleshooting

Cell culture

The following table lists some potential problems and solutions to help you troubleshoot your cell culture problems.

Problem	Cause	Solution
No viable cells after thawing stock	Stock not stored correctly	Order new stock and store in liquid nitrogen. Keep in liquid nitrogen until thawing.
	Home-made stock not viable	Freeze cells at a density of 1 × 10^7 viable cells/mL.
		Use a freezing medium composed of 90% fresh FreeStyle™ CHO™ Expression Medium and 10% DMSO.
		Use low-passage cells to make your own stocks.
		Follow the procedures in (“Cryopreservation” on page 13) exactly.
		Obtain new FreeStyle™ CHO-S™ Cells (“Accessory products” on page 22).
	Thawing medium not correct	Use FreeStyle™ CHO™ Expression Medium supplemented with 8 mM L-Glutamine (pre-warm before use).
		Do not add antibiotics to media as this may negatively impact cell growth.
	Shaker not set up properly	See “Subculture cells” on page 11 for proper settings for orbital shaker.
	Cells too diluted	Spin down culture and grow cells in a smaller culture volume.

Problem	Cause	Solution
Cells grow slowly	Growth medium not correct	Use FreeStyle™ CHO™ Expression Medium supplemented with 8 mM L-Glutamine (pre-warm before use).
		Do not add more than 5 mL/L of Penicillin/Streptomycin (0.5X Pen Strep) to media as this may impact cell growth.
	Shaker not set up properly	See “Subculture cells” on page 11 for proper settings for orbital shaker.
	Medium foamy	Lower the shaker speed slightly till no foam forms.
	Flasks too small	Use flasks that are at least 2.5 times bigger than the culture volume.
	Cells too old	Use healthy FreeStyle™ MAX CHO-S™ cells under passage 25; do not overgrow.
	Cell culture clumpy	Prevent this by sufficient agitation of the culture, a regular and frequent cell passage schedule, and maintenance of cells at recommended densities.
	Cells transfected	Cells that are transfected with high efficiency grow slower than untransfected cells. This is a good thing.

Transfection and protein production

The following table lists some potential problems and possible solutions that may help you troubleshoot your transfection and protein production experiments.

Problem	Cause	Solution
Low Transfection Efficiency and/or Low Protein Yield	Cells cultured for too many passages (≥25 passages)	Thaw a new batch of early-passage cells.
	Cells not passed 24 hours before transfection	Approximately 24 hours before transfection, pass cells at 5–6 × 10^5 cells/mL.
	Improperly cultured FreeStyle™ CHO-S™ cells	Exactly follow procedures as outlined in **Subculture cells** section ("Subculture cells" on page 11).
	Cells transfected in media containing too much antibiotics	Do not add more than 5 mL/L of Penicillin/Streptomycin (0.5X Pen Strep) to media.
	FreeStyle™ Max™ Reagent handled incorrectly	Store at 4°C. **Do not freeze.**
		Mix gently by inversion. **Do not vortex.**
	Used poor quality expression construct plasmid DNA	**Do not** use mini-prep plasmid DNA for transfection. Use a PureLink™ HiPure Plasmid Kit to prepare plasmid DNA with low endotoxin contamination.
	Suboptimal transfection conditions	Perform transfections with positive control plasmid pCMV SPORT-βgal to assess your transfection conditions.
		Assess transfection efficiency via expression of a GFP–type fluorescent protein (we recommend monitoring the cultures starting at 24 hours post-transfection.)
		Vary the amounts of DNA and FreeStyle™ MAX Reagent used (see "Optimize protein expression" on page 17).

Problem	Cause	Solution
Low Transfection Efficiency and/or Low Protein Yield	DNA not sterile	Sterilize DNA (see "Plasmid preparation" on page 15).
	Gene of interest is toxic to cells	Do not generate constructs containing activated oncogenes or harmful genes.
		Try FreeStyle™ MAX 293 Expression System.
	Protein harvested too early or too late	When expressing a protein for the first time, perform a time course experiment between days 1 and 7 post-transfection to identify the peak of protein production, and to monitor cell viability.

Appendix

Accessory products

Additional products

The products listed in this section may be used with the FreeStyle™ CHO™ Cells. For more information, refer to our website (**www.lifetechnologies.com**) or call Technical Support (see).

Item	Quantity	Catalog no.
FreeStyle™ MAX CHO™ Expression System	1 kit	K9000-20
FreeStyle™ CHO™ Expression Medium	1 L	12651-014
	6 × 1 L	12651-022
FreeStyle™ MAX Reagent	1 mL	16447-100
OptiPRO™ SFM™	100 mL	12309-050
	1000 mL	12309-019
L-Glutamine-200 mM (100X), liquid	100 mL	25030-081
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100-04
PureLink™ HiPure Plasmid Maxiprep Kit	10 preps	K2100-06
PureLink™ HiPure Plasmid Filter Maxiprep Kit	10 preps	K2100-16
PureLink™ HiPure Plasmid Megaprep Kit	4 preps	K2100-08
Trypan Blue Stain	100 mL	15250-061
FluoReporter™ *lacZ*/Galactosidase Quantitation Kit	1000 assays	F-2905
Penicillin-Streptomycin, liquid	100 mL	15140-122

FreeStyle™ MAX 293 expression system

Protein production using the FreeStyle™ MAX Expression Systems can be performed in CHO™ cells or 293 cells. Ordering information is provided for the reagents specific for the FreeStyle™ MAX 293 Expression System.

Item	Quantity	Catalog no.
FreeStyle™ MAX 293 Expression System	1 kit	K9000-10
FreeStyle™ 293-F Cells	1 vial (1×10^7 cells)	R790-07
FreeStyle™ 293 Expression Medium	1 L	12338-018
	6 × 1 L	12338-026

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Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

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  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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