FreeStyle™ F17 Expression Medium

Package Contents

Catalog Number	Size
A13835-01	1000 mL
A13835-02	6 × 1000 mL

Storage Conditions

- Store at 4°C for a 12-month shelf life.
- Protect from light.

Required Materials

- FreeStyle™ 293-F Cells or FreeStyle™ CHO-S® Cells
- GlutaMAX™-I supplement or 200 mM L-glutamine
- 125-mL polycarbonate, disposable, sterile, vent-cap Erlenmeyer shaker flask or other appropriate vessel for culturing suspension cells
- Orbital shaker in temperature and CO_2 controlled incubator
- Reagents and equipment to determine cell viability (e.g., hemocytometer with trypan blue or cell counter)

Timing

Thawing and Recovery: 2–3 days
Subculturing: Every 2–3 days

Selection Guide

Protein Expression Systems
Go online to view related products.

Product Description

- FreeStyle™ F17 Expression Medium is a chemically defined and serum-free medium specifically developed to support the growth and transfection of FreeStyle™ 293-F cells in suspension.
- This medium also supports the growth and transfection of Chinese Hamster Ovary (CHO) cells.
- This medium does not contain any proteins, hydrolysates, or components of animal origin.

Important Guidelines

- FreeStyle™ F17 Expression Medium requires supplementation with L-glutamine or GlutaMAX™-I. Aseptically add to a final concentration of 4 mM for 293 cells and 8mM for CHO prior to use.
- Subculture FreeStyle™ 293-F Cells when they reach a density of approximately 1–3 × 10^6 viable cells/mL, typically every 2–3 days. Split the culture to 0.2–0.5 × 10^6 cells.
- Keep cell densities between 1–3 × 10^6 cells/mL of culture for best performance.

Online Resources

Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support.

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

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Print Options

Protocol Outline

A. Thaw cells.
B. Passage cells every 2–3 days.

Recovery and Subculturing Protocol

See page 2 to view a typical recovery and subculturing procedure.

Scaling Up FreeStyle™ Cell Culture

You can scale up FreeStyle™293-F cultures in spinner flasks or bioreactors. Determine the optimum spinner or impeller speed and seeding density based on your culture system.

If the split ratio of cells to fresh media is less than 1:2, then centrifuge the cell suspension and resuspend the cell pellet in fresh, pre-warmed FreeStyle™ F17 Expression Medium prior to inoculating the spinner or bioreactor culture.

At high stirring speeds (i.e. greater than 130 rpm) and/or depending on the impeller design, you may need to supplement the FreeStyle™ F17 Expression Medium with additional Pluronic® F-68 to avoid shear stress in the culture.

Adapting Cells to FreeStyle™ F17 Expression Medium

Cryopreserving FreeStyle™ Cells

Limited Product Warranty and Disclaimer Details

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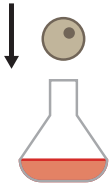
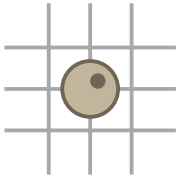
Thawing and Culturing FreeStyle™ Cells in FreeStyle™ F17 Expression Medium

Follow the procedure below to recover and passage FreeStyle™ Cells in FreeStyle™ F17 Expression Medium.

Timeline		Steps	Procedure Details
Day 1	1 	**Thaw cells**	Rapidly thaw the cells in a water bath, decontaminate the vial using 70% ethanol, and open the cryovial in a class II biological cabinet.
	2 	**Add cells to medium**	Add cells to 29 mL of pre-warmed medium in 125-mL shake flask.
	3 	**Count cells and determine viability**	Within 1–2 hours post-thaw, count cells and determine viability. Use hemocytometer and trypan blue exclusion method or automated cell counter. Cell density should be approximately 0.3 × 10^6 cells/mL and cell viability >90%.
	4 	**Incubate**	**Temperature** 37°C | **Humidified Atmosphere** 8% CO_2 in air | **Orbital Shaker Platform** 125 rpm
Days 2–4	5 	**Subculture cells**	**First passage:** When cell density reaches >1 × 10^6 cells/mL at ≥ 90% viability (typically 2–5 days post-thaw), split cells to 0.2–0.5 × 10^6 cells/mL in FreeStyle™ F17 medium. **Subsequent passages:** Every 2–3 days, cells should reach 1–3 × 10^6. Split to 0.2–0.5 × 10^6 cells/mL. Do not grow above 3 × 10^6 cells/mL. We recommend using a 125- or 250-mL flask containing 30–60 mL of medium, respectively.