FreeStyle™ 293 Expression Medium

Package Contents

Catalog Number	Size
12338-018	1000 mL
12338-026	6 × 1000 mL
12338-001	10 L
12338-002	20 L

Storage Conditions

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

Required Materials

- FreeStyle™ 293-F Cells
- 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™ 293 Expression Medium is a chemically defined and serum-free medium, specifically developed to support the growth and transfection of FreeStyle™ 293-F Cells under suspension culture conditions.
- This medium does not contain any proteins, hydrolysates, or components of animal origin.

Important Guidelines

- FreeStyle™ 293 Expression Medium contains GlutaMAX™-I supplement and does not require supplementation with L-glutamine or GlutaMAX™-I supplement.
- 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 FreeStyle™ 293-F culture to 0.2–0.5 × 10^6 cells/mL.
- 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.

FreeStyle™ 293-F Cell Culturing Protocol

See page 3 to view a typical procedure for subculturing.

Scaling Up FreeStyle™ 293-F Cell Culture

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

If the split ratio of cells to fresh media is less than 1:2, you may need to spin down the cell suspension and resuspend in fresh, pre-warmed FreeStyle™ 293 Expression Medium prior to inoculating the spinner or bioreactor culture.

At high stirring speeds (i.e. >130 rpm) and/or depending on the impeller design, you may need to supplement the FreeStyle™ 293 Expression Medium with additional Pluronic® F-68 (2.5–5 mL/L of 10% Pluronic® F-68) to avoid shear stress in the culture.

Adapting Other 293 Cells to FreeStyle™ 293 Expression Medium

Cryopreserving FreeStyle™ 293-F Cells

Limited Product Warranty and Disclaimer Details

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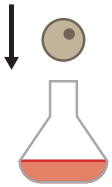
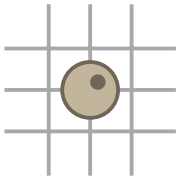
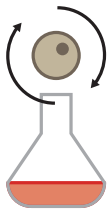
Thawing and Passaging FreeStyle™ 293-F Cells in FreeStyle™ 293 Medium

Follow the procedure below to recover and subculture FreeStyle™ 293-F Cells in FreeStyle™ 293 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 3–4	5 	**Subculture cells**	**First passage:** When cell density reaches >1 × 10^6 cells/mL at ≥ 90% viability (typically 2–3 days post-thaw), split cells to 0.3 × 10^6 cells/mL in FreeStyle™ 293 Expression 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 or 60 mL of medium, respectively.