FreeStyle™ 293-F Cells

Catalog Number R79007

Pub. No. MAN0007834 Rev. C.0

WARNING! 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 **thermofisher.com/support**.

Product description

Gibco™ FreeStyle™ 293-F Cells are derived from the 293 cell line and are intended for use with the FreeStyle™ MAX 293 Expression System or FreeStyle™ 293 Expression System.

FreeStyle™ 293-F Cells can be thawed, grown, maintained, and transfected in FreeStyle™ 293 Expression System.

Contents and storage

Contents	Cat. No.	Amount	Storage
FreeStyle™ 293-F Cells	R79007	1 vial (1×10^7 cells)	Liquid nitrogen. Protect cultures from light.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
FreeStyle™ 293 Expression Medium	12338018
125-mL polycarbonate, disposable, sterile, vent-cap Erlenmeyer shaker flask or other appropriate vessel for culturing suspension cells	MLS
Orbital shaker in temperature and CO_2 controlled incubator	MLS
Reagents and equipment to determine cell viability (e.g., hemocytometer with trypn blue or cell counter)	MLS

Procedural guidelines

- Subculture the FreeStyle™ 293-F Cells a minimum of three times to allow them to recover from thawing before using them in transfection experiments.
- Keep cell densities between 1×10^6–3×10^6 cells/mL of culture for best performance.
- We recommend maintaining cells in a 125-mL or a 250-mL polycarbonate, disposable, sterile Erlenmeyer flask containing 25–40 mL or 50–80 mL total working volume of cell suspension, respectively.
- Glass flasks without baffles may be used, but clean them thoroughly after each use to avoid potential toxicity.

Guidelines for thawing and storing cells

- On receipt, either thaw the cells immediately or immediately place the frozen cells 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 following receipt on dry ice, allow the cells to remain in liquid nitrogen for 3–4 days before thaw.
- Before starting experiments, ensure to have cells that are established and have frozen stocks on hand. On receipt, grow and freeze multiple vials of cells to ensure that you have an adequate supply of early-passage cells.

FreeStyle™ 293-F Cells characteristics

Growth properties: Suspension

Doubling time: 25 hours. Doubling times may vary based on cell health, handling, and passage number.

Viability during log phase culture: >90%

Subculture conditions: Grow to 1×10^6–3×10^6 cells/mL, and split cells to 0.2×10^6–0.5×10^6 cells/mL every 2–3 days. Do not grow above above 3×10^6 cells/mL for best performance. Discard cells when they reach passage number 30.

Scale up FreeStyle™ 293-F cell culture

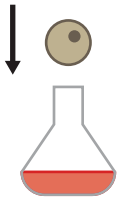
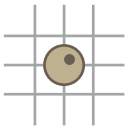
You can scale up the FreeStyle™ 293-F cultures in spinner flasks or bioreactors. Determine the optimal spinner or impeller speed and seeding density for your culture system. We recommend that the cells be seeded at 0.5×10^6 viable cells/mL. Optimum spinner speed is approximately 100–130 rpm, and optimum impeller speed in Celligen™ stirred tank bioreactors is 70–100 rpm. If the split ratio of cells to fresh media is less than 1:2, centrifuge the cell suspension and re-suspend the cell pellet in fresh medium before inoculating the culture.

Cryopreserve FreeStyle™ 293-F Cells

FreeStyle™ 293-F Cells can be frozen directly in FreeStyle™ 293 Expression Medium.

- Freeze FreeStyle™ 293-F Cells at a final density of 1×10^7 viable cells/mL.
- Use a freezing medium composed of 90% fresh FreeStyle™ 293 Expression Medium and 10% DMSO.
- Freeze cells in an automated or manual, controlled-rate, freezing apparatus following standard procedures. For ideal cryopreservation, the rate of temperature decrease should be 1°C per minute.
- Transfer frozen vials to liquid nitrogen for storage.
- Check the viability and recovery of frozen cells 24 hours after storing cryovials in liquid nitrogen by following the procedures outlines in this protocol.

Thaw and passage FreeStyle™ 293-F Cells in FreeStyle™ 293 Expression Medium

1	**Day 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	**Day 1**: Add cells to medium 	Add cells to 29 mL of pre-warmed medium in a 125-mL shake flask.
3	**Day 1**: 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	**Day 1**: Incubate 	**Temperature**: 37°C **Humidified atmosphere**: 8% CO_2 in air **Orbital shaker platform**: 125 rpm
5	**Day 3–4**: Subculture cells 	**First passage**: When cell density reaches >1 × 10^6 cells/mL at ≥90% viability (typically 2–3 days post-thaw), split the culture to 0.2 × 10^6–0.5 × 10^6 cells/mL in FreeStyle™ 293 Expression Medium. **Subsequent passages**: Every 2–3 days, cells should reach 1 × 10^6–3 × 10^6 cells/mL. Split to 0.2 × 10^6–0.5 × 10^6 cells/mL. Do not grow above 3 × 10^6 cells/mL. We recommend using a 125-mL or a 250-mL flask containing 25–40 mL or 50–80 mL of medium, respectively.: When cell density reaches > 1 × 10^6 cells/mL at ≥90% viability (typically 2–3 days post-thaw), split the culture to 0.2 × 10^6–0.5 × 10^6 cells/mL in FreeStyle™ 293 Expression Medium.

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 at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Corporation | 5781 Van Allen Way | Carlsbad, CA 92008
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.