FreeStyle™ MAX 293 Expression System

Package Contents	**Catalog Number:** K9000-10	**Amounts**
	▪ FreeStyle™ 293-F Cells	1 mL
	▪ FreeStyle™ MAX Reagent	1 mL
	▪ FreeStyle™ 293 Expression Medium	1 Liter
	▪ OptiPRO™ SFM	100 mL
	▪ pCMV SPORT-βgal	25 µg
Storage Conditions	▪ Store cells in liquid nitrogen. ▪ Store reagent and media at 4°C. ▪ Protect media from light. ▪ Store the control vector at –20°C.	
Required Materials	▪ 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	
Timing	Thawing and Recovery: 2–3 days Subculturing: Every 2–3 days Transfection: 1–7 days	
Selection Guide	Protein Expression Systems Go online to view related products.	
Product Description	▪ The FreeStyle™ MAX 293 Expression System facilitates large-scale transfection of suspension 293 human embryonic kidney cells, in a defined, serum-free medium, for expression of proteins and virus. ▪ Transfection and expression experiments may be performed directly in FreeStyle™ 293 Expression Medium without the need for media change. ▪ The kit provides enough reagents to perform 25 transfections and one control transfection in a 30-mL volume. ▪ All reagents are completely animal origin-free, including the defined, serum-free medium, which may be imperative for regulatory requirements.	
Important Guidelines	General Cell Handling Preparing Media	
Online Resources	Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support.	

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Print Options

Protocol Outline

A. Thaw cells.
B. Subculture cells.
C. Transfect cells and generate protein or virus.

FreeStyle™ MAX 293 Expression System Kit Characteristics

- 293-F cell-based system
- High yields in 1 to 7 days
- Scalable from multi-well plates to liter scale

FreeStyle™ MAX 293 Expression System Individual Components

The FreeStyle™ MAX 293 Expression System includes the following major components:

Click the ⓘ next to each product to go to its specific protocol.

ⓘ **FreeStyle™ 293-F Cells:** This cell line is adapted to high density, serum-free, suspension growth and maintained in FreeStyle™ 293 Expression Medium. These cells show high transfection efficiencies with FreeStyle™ MAX Reagent.

ⓘ **FreeStyle™ 293 Expression Medium:** This medium is an optimized, serum-free and protein-free formulation, designed to support the high-density culture and transfection of FreeStyle™ 293-F Cells in suspension.

ⓘ **FreeStyle™ MAX Reagent**: This transfection reagent provides high transfection efficiency in suspension FreeStyle™ 293-F Cells.

ⓘ Limited Product Warranty and Disclaimer Details

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FreeStyle™ 293-F Cells

	Catalog Numbers	Size
Package Contents	R790-07	1 vial containing 1 × 10^7 cells

Storage Conditions
- Store in liquid nitrogen.
- Protect cultures from light.

Required Materials
- FreeStyle™ 293 Expression Medium
- 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
- The FreeStyle™ 293-F cell line is derived from the 293 cell line and is 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 Medium.

Important 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 1–3 × 10^6 cells/mL of culture for best performance.
- We recommend maintaining cells in a 125-mL or 250-mL polycarbonate, disposable, sterile Erlenmeyer flask containing 25–40 mL or 50–80 mL total working volume of cell suspension, respectively.
- Glass flasks may be used, but clean them thoroughly after each use to avoid potential toxicity.

Online Resources
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Protocol Outline

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

Invitrogen FreeStyle™ 293-F Cells Protocol

See page 3 to view a typical procedure for thawing and culturing 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 1–3 × 10^6 cells/mL, and split cells to 0.2–0.5 × 10^6 cells/mL, every 2–3 days. Do not grow above 3 × 10^6 cells/mL for best performance. Discard cells when they reach passage number 30.

Scaling 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 0.2–0.5 × 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.

Cryopreserving FreeStyle™ 293-F Cells

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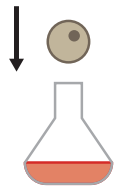
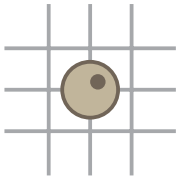
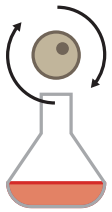
Thawing and Passaging FreeStyle™ 293-F Cells in FreeStyle™ 293 Expression Medium

Follow the procedure below to recover and subculture FreeStyle™ 293-F Cells.

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 $>1 \times 10^6$ cells/mL at ≥ 90% viability (typically 2–3 days post-thaw), split cells to $0.2–0.5 \times 10^6$ cells/mL in FreeStyle™ 293 Expression Medium. **Subsequent passages:** Every 2–3 days, cells should reach $1–3 \times 10^6$. Split to $0.2–0.5 \times 10^6$ 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.

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.

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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 5 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

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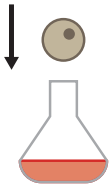
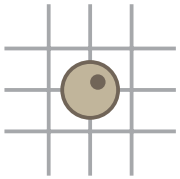
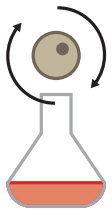
Thawing and Passaging FreeStyle™ 293-F Cells in FreeStyle™ 293 Medium

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.

FreeStyle™ MAX Reagent

Package Contents

Catalog Number	Size
16447-100	1.0 mL
16447-500	15.0 mL
16447-750	10 × 15.0 mL

Storage Conditions

- Store at 4°C.
- Do not freeze.

Required Materials

- FreeStyle™ 293-F Cells, FreeStyle™ CHO-S® Cells, or DG44 Cells
- FreeStyle™ 293 Expression Medium, FreeStyle™ CHO Expression Medium, or DG44 Medium
- Erlenmeyer flasks with vented caps
- Orbital shaker in temperature and CO_2 controlled incubator
- Plasmid DNA
- OptiPRO™ SFM

Timing

Cell Preparation: 1 day
Transfection: 10–20 minutes

Selection Guide

Protein Expression Systems
Go online to view related products.

Product Description

- FreeStyle™ MAX Reagent is a proprietary, animal origin-free formulation for transfecting plasmid DNA into eukaryotic cells, which can be easily scaled up to produce large amounts of recombinant proteins.
- This transfection reagent is formulated specifically for use with FreeStyle™ 293-F, FreeStyle™ CHO-S®, and DG44 cells.

Important Guidelines

- DNA-FreeStyle™ MAX complexes must be made in OptiPRO™ SFM and can be added directly to cells in culture medium.
- Cultivate FreeStyle™ 293-F and FreeStyle™ CHO-S® Cells, or DG44 Cells, in a humidified, 37°C, 8% CO_2 environment in suspension on an orbital shaker.

Online Resources

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

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Print Options

Protocol Outline

A. Culture cells at least three passages after thawing.
B. Prepare and add DNA-lipid complexes to cells.
C. Incubate cells for 1–7 days.
D. Harvest.

Transfection Protocol

See page 7 to view a typical procedure for transfecting FreeStyle™ 293-F and FreeStyle™ CHO-S® Cells for protein expression.

See page 8 to view a typical procedure for transfecting DG44 cells to generate stable cell lines.

Transfection Conditions for FreeStyle™ Cells

Final transfection volume: 30 mL
Number of cells to transfect: 3×10^7
Amount of plasmid DNA: 37.5 µg
Amount of FreeStyle™ MAX Reagent: 37.5 µL

Scaling Up or Down Transfections

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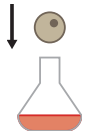
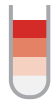
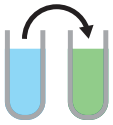
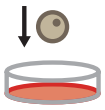
Transfecting FreeStyle™ 293-F or FreeStyle™ CHO-S® Cells

Use the following protocol to transfect suspension cells. All amounts are given on a per-flask basis for 30-mL cultures in 125-mL shake flasks.

Timeline		Steps	Procedure Details
Day -1	1 	**Expand cells**	For each 30-mL transfection, you will need 3×10^7 cells in 30 mL of FreeStyle™ 293 Expression Medium or FreeStyle™ CHO Expression Medium. **For FreeStyle™ 293-F Cells:** One day prior to transfection, passage at $6–7 \times 10^5$ cells/mL; shake at 120–135 rpm. **For FreeStyle™ CHO-S® Cells:** One day prior to transfection, passage at $5–6 \times 10^5$ cells/mL; shake at 120–135 rpm.
	2 	**Count cells and determine viability**	Use the trypan blue dye exclusion method to determine cell viability and clumping in a small aliquot of cells. Use an automated cell counter or a hemocytometer to determine cell counts. On the day of transfection, your cells should have a density of $1.2–1.5 \times 10^6$ cells/mL at >95% viability.
	3 	**Seed cells in flask**	Dilute cells to 1×10^6 cells/mL. You will need 3×10^7 cells for each 30-mL transfection. Use fresh, pre-warmed FreeStyle™ 293 Expression Medium or FreeStyle™ CHO Expression Medium to a total volume of 30 mL for each 30-mL transfection.
Day 0	4 	**Prepare DNA-lipid complexes**	Prepare DNA-lipid complexes as follows: a. Dilute 37.5 µg of plasmid DNA in OptiPRO™ SFM reduced serum medium to a total volume of 0.6 mL. Mix gently. b. Dilute 37.5 µL of FreeStyle™ MAX Reagent in OptiPRO™ SFM reduced serum medium to a total volume of 0.6 mL. Mix gently and incubate for 5 minutes at room temperature. Incubation times longer than five minutes may result in decreased activity. c. After the 5-minute incubation, add the diluted DNA to the diluted reagent to obtain a total volume of 1.3 mL. Mix gently. d. Incubate for 20–30 minutes at room temperature to allow the DNA-lipid complexes to form.
	5 	**Add DNA-lipid complex to cells**	Add 1.2 mL of complex to each cell suspension flask. Each flask should have a total volume of 30 mL, and contain approximately 1×10^6 viable cells/mL. To the negative control flask, add 2 mL of reduced serum medium instead of complex.
	6 	**Incubate**	**Temperature** 37°C | **Humidified Atmosphere** 8% CO_2 in air | **Orbital Shaker Platform** 125 rpm
Days 1–7	7 	**Harvest cells or media**	Assay for recombinant protein expression. Perform this step 1–7 days post-transfection. Harvest media instead of cells if recombinant protein is secreted.

Transfecting DG44 Cells to Generate Stable Cell Lines

Use this procedure to transfect linearized DNA into DG44 cells. All amounts are on a per-flask basis for 30-mL cultures in 125-mL shake flasks.

Timeline		Steps	Procedure Details
Day 0	1 	**Prepare and culture the DG44 cells**	a. Passage the cells at 3 × 10^5 cell/mL. b. Shake at 130–135 rpm at 37°C, 8% CO_2. c. Culture in CD DG44 Medium (Cat. No. 12610-010) with 8 mM L-glutamine (Cat. No. 25030-081) and 18 mL/L of 10% Pluronic® F-68 (Cat. No. 24040-032).
Day 1	2 	**Passage the DG44 cells again**	Passage cells again at 3 × 10^5 cell/mL.
Day 2	3 	**Prepare the cells**	Count the cells. Cell viability should be >95%. In each flask, add 1.5 × 10^7 cells in a total volume of 30 mL CD DG44 Medium.
	4 	**Combine lipid and linearized DNA**	Gently invert the tube to mix the reagent. Then, add 18 µg of linearized DNA and 15 µg of FreeStyle™ MAX Reagent into 1.2 mL of OptiPRO™ SFM (at room temperature), and gently invert to mix.
	5 	**Incubate the DNA-lipid mixture**	Incubate for 10 minutes at room temperature, but no longer than 20 minutes.
	6 	**Add DNA-lipid mixture to cells**	Slowly add 1.2 mL of mixture into the 125-mL flask containing the cells while slowly swirling the flask.
	7 	**Incubate**	**Temperature** 37°C **Humidified Atmosphere** 8% CO_2 in air **Orbital Shaker Platform** 130–135 rpm
Day 4	8 	**Place cells on a selective medium**	Place cells on a selective medium (for example, CD OptiCHO™ Medium, Cat. No. 12681-011).