FreeStyle™ MAX Reagent

USER GUIDE

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gibco

Print Options

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.thermofisher.com/support.

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 2 to view a typical procedure for transfecting FreeStyle™ 293-F and FreeStyle™ CHO-S™ Cells for protein expression.

See page 3 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

Limited Product Warranty and Disclaimer Details

ThermoFisher SCIENTIFIC

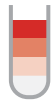
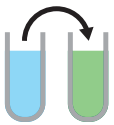
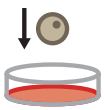
Transfecting FreeStyle™ 293-F or FreeStyle™ CHO-S™ Cells

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).