293fectin™ Transfection Reagent

USER GUIDE

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gibco

Print Options

Package Contents

Catalog Number	Size
12347-019	1 mL
12347-500	15 mL
12347-750	10 × 15 mL

Storage Conditions

- Store at 4°C.
- Do not freeze.

Required Materials

- Plasmid DNA
- 125-mL polycarbonate, vent-cap Erlenmeyer shaker flask or similar
- Orbital shaker in temperature- and CO_2-controlled incubator
- FreeStyle™ 293-F cells
- FreeStyle™ 293 Expression Medium
- Opti-MEM® Reduced Serum Medium

Timing

Advance planning: 1 day
Transfection: 30–40 minutes
Incubation: 2–7 days

Selection Guide

Transfection Reagents
Go online to view related products.

Product Description

- 293fectin™ Reagent is a proprietary, cationic lipid-based formulation for transfecting DNA into eukaryotic cells.
- This reagent is optimized for transfecting suspension 293 human embryonic kidney cells in defined, serum-free FreeStyle™ 293 Expression Medium, and is intended for use with the FreeStyle™ 293 Expression System.

Important Guidelines

- DNA-293fectin™ complexes must be made in serum-free medium such as Opti-MEM® Reduced Serum Medium and can be added directly to cells in culture medium.
- Make sure your plasmid DNA is clean, sterile, and free from phenol and sodium chloride. Contaminants may kill the cells, salt will interfere with complexing, and both will decrease transfection efficiency. We recommend isolating plasmid DNA using one of the PureLink® HiPure Plasmid Kits (Cat. no. K2100-14 or K2100-16).

Online Resources

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

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

293fectin™ Transfection Reagent Protocol

See page 2 to view a typical procedure for transfecting suspension cells.

Recommended Conditions for Transfection

To transfect suspension 293 cells in FreeStyle™ 293 Expression Medium, use the following optimized conditions. To perform transfection experiments in a larger volume, simply scale up the volume of reagents accordingly.

- **Final transfection volume:** 30 mL
- **Number of cells to transfect:** 3×10^7 cells (final cell density: 1×10^6 cells/mL) cultured in FreeStyle™ 293 Expression Medium. Make sure that the cells are healthy and greater than 90% viable before proceeding to transfection.
- **Amount of plasmid DNA:** 30 µg
- **Amount of 293fectin™ Reagent:** 60 µL. Use 2 µL 293fectin™ Reagent per 1 µg of plasmid DNA transfected.

Optimizing Protein Expression

Expression levels may vary depending on the nature of your recombinant protein. Perform a time course experiment by harvesting cells or media at 2–7 days post-transfection to optimize expression of your recombinant protein.

Limited Product Warranty and Disclaimer Details

Limited Use Label License

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Transfecting Suspension 293 Cells

Use the following protocol to transfect suspension cells.

Timeline	Steps	Procedure Details
Day 0 1 	**Expand cells**	For each 30-mL transfection, you will need 3×10^7 cells in 28 mL of FreeStyle™ 293 Expression Medium. Expand cells accordingly, taking into account the cell doubling time. For FreeStyle™ 293-F cells, this equates to passaging cells at ~6–7 $\times 10^5$ cells/mL.
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 a Coulter Counter or a hemocytometer to determine cell counts. The viability of cells must be over 90%. For best results, make make sure to have a single-cell suspension. You may need to vortex the cells vigorously for 10–45 seconds to break up cell clumps.
Day 1 3 	**Seed cells in flask**	Calculate the volume of cell suspension containing the number of cells needed for one transfection. You will need 3×10^7 cells for each 30-mL transfection. Use fresh, pre-warmed FreeStyle™ 293 Expression Medium to a total volume of 28 mL for each 30-mL transfection. Use sterile, disposable 125-mL Erlenmeyer shaker flasks for this step.
4 	**Prepare Lipid-DNA complexes**	Prepare lipid-DNA complexes as follows: a. Dilute 30 µg of plasmid DNA in Opti-MEM® I reduced serum medium to a total volume of 1 mL. Mix gently. b. Dilute 60 µL of 293fectin™ reagent in Opti-MEM® I reduced serum medium to a total volume of 1 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 2 mL. Mix gently. d. Incubate for 20–30 minutes at room temperature to allow the DNA-reagent complexes to form.
5 	**Add lipid-DNA complex to cells**	Add 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 the cells**	**Temperature** 37°C **Humidified Atmosphere** 8% CO_2 in air **Orbital Shaker Platform** 125 rpm
Day 2–7 7 	**Harvest cells or media**	Assay for recombinant protein expression. Perform this step at approximately 2–7 days post-transfection. Harvest media instead of cells if recombinant protein is secreted.