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293fectin™ Transfection Reagent

Part no. 100004835 MAN0000775 **Rev. Date** 7 June 2011

Cat. nos.:	**Size:**	**Store at 4°C (do not freeze)**
12347-019	1 mL	
12347-500	15 mL	
12347-750	10 × 15 mL	

Description

293fectin™ Reagent is a proprietary, cationic lipid-based formulation for transfecting DNA into eukaryotic cells. 293fectin™ Reagent is optimized for transfecting suspension 293 human embryonic kidney cells (e.g. FreeStyle™ 293-F cells, Cat. no. R790-07) in defined, serum-free FreeStyle™ 293 Expression Medium (Cat. no. 12338-018), and is intended for use with the FreeStyle™ 293 Expression System (Cat. no. K9000-01).

Advantages

Using 293fectin™ Reagent provides the following advantages:

- 293fectin™ Reagent demonstrates high transfection efficiency in suspension 293 cells, and is also suitable for transfecting adherent 293 cells.
- Suspension FreeStyle™ 293-F cells may be transfected in FreeStyle™ 293 Expression Medium; no medium change is required.
- Add 293fectin™ Reagent complexes directly to cells in culture medium.
- It is not necessary to remove complexes or change or add medium following transfection.

Important Guidelines for Transfection

- For optimal transfection efficiency, dilute 293fectin™ Reagent in Opti-MEM® I Reduced Serum Medium (Cat. no. 31985-062) prior to complexing with DNA.
- Make sure your plasmid DNA is clean, sterile, and free from phenol and sodium chloride. Contaminants may kill the cells, and salt will interfere with complexing, both of which will decrease transfection efficiency. We recommend isolating plasmid DNA using one of the PureLink® HiPure Plasmid Kits (Cat. no. K2100-14 or K2100-16).

Intended Use: For research use only.
Not intended for any animal or human therapeutic or diagnostic use.

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:** 20–40 µg (we typically use 30 µg).
- **Amount of 293fectin™ Reagent:** 40–80 µL (we typically use 60 µL). Use 2 µL 293fectin™ Reagent per 1 µg of plasmid DNA transfected.

Transfecting Suspension Cells

Use the following protocol to transfect suspension 293 cells in a **30 mL volume**. You may keep the cells in FreeStyle™ 293 Expression Medium during transfection. Do not add selection antibiotics to media during transfection, as this may decrease transfection efficiency. Include a positive control and a negative control (no DNA, no 293fectin™ Reagent) to help you evaluate results.

1. The day before transfection, determine the number of cells needed for transfection. 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 passing cells at ~6–7 $\times 10^5$ cells/mL.
2. On the day of transfection, determine the viability and the amount of cell clumping from a small aliquot of cells using the trypan blue dye exclusion method. Vigorously vortex for 45 seconds to break up cell clumps and determine total cell counts using a Coulter Counter or a hemacytometer. The viability of cells must be over 90%.

   Important: For best results, make sure to have a single-cell suspension. It may be necessary to vortex the cells vigorously for 10–30 seconds to break up cell clumps.
3. 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). Place the shaker flask containing cells in a 37°C incubator on an orbital shaker.

Transfecting Suspension Cells, Continued

4. For each transfection sample, prepare lipid-DNA complexes as follows:
   a. Dilute 30 µg of plasmid DNA in Opti-MEM® I to a total volume of 1 mL. Mix gently.
   b. Dilute 60 µL of 293fectin™ Reagent in Opti-MEM® I to a total volume of 1 mL. Mix gently and incubate for 5 minutes at room temperature.

      Note: Longer incubation times may result in decreased activity.

   c. After the 5 minute incubation, add the diluted DNA to the diluted 293fectin™ Reagent to obtain a total volume of 2 mL. Mix gently.
   d. Incubate for 20–30 minutes at room temperature to allow the DNA-293fectin™ complexes to form.
5. While the DNA-293fectin™ complexes are incubating, remove the cell suspension from the incubator and add the appropriate volume of cell suspension (see step 3 of this procedure) into a sterile, disposable 125-mL Erlenmeyer shaker flask. Add fresh, **pre-warmed** FreeStyle™ 293 Expression Medium to a total volume of 28 mL for each 30-mL transfection.
6. After the DNA-293fectin™ Reagent incubation is complete, add the 2 mL of DNA-293fectin™ complex (from step 4 of this procedure) to each shaker flask containing the cell suspension. To the negative control flask, add 2 mL of Opti-MEM® I instead of DNA-293fectin™ complex. Each flask should have a total volume of 30 mL, and contain approximately 1×10^6 viable cells per mL.
7. Incubate the cells in a 37°C incubator with a humidified atmosphere of 8% CO_2 in air on an orbital shaker rotating at 125 rpm.
8. Harvest cells or media (if recombinant protein is secreted) at approximately 48 hours post-transfection and assay for recombinant protein expression.

Transfecting Adherent Cells

Use the following protocol to transfect adherent 293 cells in **24-well** plates. If you are using larger- or smaller-sized tissue culture plates, vary the transfection conditions (i.e. seeding density, amount of DNA, 293fectin™ Reagent, and medium) in proportion to the difference in surface area.

1. The day before transfection, trypsinize and count the cells. Plate the cells at a density of 2×10^5 cells/well so that they are 90–95% confluent on the day of transfection. Plate cells in 0.5 mL of their normal growth medium containing serum. **Do not** use selection antibiotics.

Transfecting Adherent Cells, Continued

2. On the day of transfection, prepare lipid-DNA complexes for each sample as follows:
   a. Dilute 0.8–1.0 µg of plasmid DNA in Opti-MEM® I to a total volume of 50 µL. Mix gently.
   b. Dilute 2–3 µL of 293fectin™ Reagent in Opti-MEM® I to a total volume of 50 µL. Mix gently and incubate for 5 minutes at room temperature.

      Note: Longer incubation times may result in decreased activity.

   c. After the 5 minute incubation, add the diluted DNA to the diluted 293fectin™ Reagent to obtain a total volume of 100 µL. Mix gently.
   d. Incubate for 20–30 minutes at room temperature to allow DNA-293fectin™ complexes to form.
3. Add 100 µL of the DNA-293fectin™ complex to each well and mix gently by rocking the plate back and forth.
4. Incubate the cells in a 37°C incubator for 24–48 hours before assaying for recombinant protein expression.

Optimizing Protein Expression

Expression levels may vary depending on the nature of your recombinant protein; therefore, perform a time course experiment by harvesting cells or media at 24, 48, 72, 96 hours post-transfection to optimize expression of your recombinant protein.

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