SignaGen® Laboratories

CalFectin™ Mammalian DNA Transfection Reagent

A Protocol for Transfecting Mammalian Cells

SKU # SL100478 | SignaGen Laboratories | For Research Use Only — Not for Diagnostic Purposes

Introduction

CalFectin™ Mammalian DNA Transfection Reagent is a chemically optimized calcium phosphate-based transfection system engineered to overcome the principal limitations of classical calcium phosphate co-precipitation: inconsistent precipitate particle size, elevated cytotoxicity, and sensitivity to serum. By employing a proprietary reduced-calcium formulation, CalFectin™ generates uniformly sized DNA–calcium complexes that promote efficient endocytosis while substantially reducing osmotic and ionic stress on target cells. The reagent supports both transient expression and the generation of stable integrant cell lines in most adherent mammalian cell types. CalFectin™ demonstrates particularly high efficiency in cell lines refractory to cationic lipid- and polymer-based methods, including HepG2, HEK293, CHO, HeLa, MDCK, COS-7, 3T3, LNCaP, C6, PC12, and primary cultured cells.

Kit Contents

Component	Volume	Storage
CalFectin™ Mammalian DNA Transfection Reagent	1.0 mL	+4 °C

Shelf life: CalFectin™ Reagent is stable for up to 24 months at +4 °C after receipt.

General Guidelines

- For optimal CalFectin™/DNA complex particle size and uniform co-precipitate formation, dilute both plasmid DNA and CalFectin™ Reagent in serum-free tissue culture medium (e.g., DMEM with high glucose). Avoid medium containing serum during the complex formation step, as serum proteins interfere with DNA–calcium complex assembly.
- The optimal CalFectin™ (µL) : DNA (µg) volumetric ratio is cell-type dependent (range 2:1–3:1 v/w). A ratio of 3:1 is recommended as an initial starting point; optimization may be required for specific cell lines or target constructs.
- Once the CalFectin™/DNA complex is formed, transfection may be carried out in complete medium containing serum (>5%) and antibiotics; these components do not significantly impair CalFectin™-mediated transfection efficiency.
- To minimize cytotoxicity, remove CalFectin™/DNA complex-containing medium and replace with fresh complete serum/antibiotic-containing medium ~18 hours post-transfection.

Procedures for Transfecting Adherent Cells

Step 1 — Cell Seeding

Seed cells 18–24 hours prior to transfection so that the monolayer reaches approximately 70% confluency at the time of transfection. Actively dividing, sub-confluent monolayers exhibit higher endocytic activity and are critical for optimal uptake efficiency. Refresh each well with complete culture medium (containing serum and antibiotics) 30–60 minutes before adding the transfection complex. Refer to **Table 1** for recommended seeding densities by culture format.

■***Note:*** *Avoid cell densities above 90% confluency at the time of transfection, as over-confluent monolayers exhibit reduced endocytic activity and substantially lower transgene expression. Serum and antibiotics in the culture medium do not significantly inhibit CalFectin™-mediated transfection.*

Table 1. Cell Seeding Guideline for Different Culture Formats

Culture Dish	Surface Area (cm^2)	Cells to Seed
100 mm Dish	58	$2.2–4.4 \times 10^6$
60 mm Dish	21	$0.9–1.8 \times 10^6$
35 mm Dish	9.6	$3.5–7.0 \times 10^5$
6-well Plate	9.6	$4.0–8.0 \times 10^5$
12-well Plate	3.5	$1.5–3.0 \times 10^5$
24-well Plate	1.9	$0.8–1.6 \times 10^5$
48-well Plate	1.0	$4.0–8.0 \times 10^4$

Culture Dish	Surface Area (cm^2)	Cells to Seed
96-well Plate	0.3	$1.2–2.4 \times 10^4$

Step 2 — Preparation of CalFectin™/DNA Complex and Transfection

The following protocol is optimized for transfection in a 24-well plate format. Refer to **Table 2** for scaling to other culture vessel formats. All volumes should be scaled proportionally.

1. (Optional) Aspirate existing medium from each well and replace with 1.0 mL of fresh complete medium containing serum and antibiotics 30–60 minutes prior to transfection.
2. Dilute 0.5 µg of plasmid DNA into 50 µL of serum-free tissue culture medium (e.g., serum-free DMEM with high glucose). Pipette up and down 3–4 times to ensure thorough mixing.
3. Add 1.5 µL of CalFectin™ Reagent directly into the diluted DNA solution. Pipette up and down 3–4 times to ensure thorough mixing.
4. Incubate the CalFectin™/DNA mixture for 10–15 minutes at room temperature (18–25°C) to allow CalFectin™/DNA complex formation.
5. Add the entire CalFectin™/DNA complex dropwise to the medium in each well. Distribute evenly by gently swirling the plate. Return the plate to the 37 ºC / 5% CO_2 incubator immediately.
6. To minimize cytotoxicity, aspirate the CalFectin™/DNA complex-containing medium ~18 hours post-transfection and replace with fresh complete serum/antibiotic-containing medium.
7. Assess transfection efficiency 24–48 hours post-transfection by appropriate reporter assay (e.g., fluorescence microscopy, flow cytometry, or luminometry). A 48-hour time point is recommended for maximum transgene expression, as protein accumulation continues beyond the initial 24-hour window.

CRITICAL: Do not incubate the CalFectin™/DNA complex for more than 20 minutes before use. Prolonged incubation results in uncontrolled particle aggregation, leading to reduced cellular uptake, diminished transfection efficiency, and increased cytotoxicity.

Note: *Use only serum-free DMEM (high glucose) or an equivalent protein-free, serum-free tissue culture medium as the diluent for complex formation. Do not use Opti-MEM I Reduced Serum Medium; although labeled reduced-serum, it contains BSA and human transferrin — serum-derived proteins that chelate calcium ions and interfere with DNA–calcium co-precipitate assembly, reducing complex formation efficiency. Standard complete medium containing serum must likewise not be used during this step.*

Table 2. Recommended Reagent Amounts for Different Culture Vessel Formats

Culture Dish	Culture Medium Volume (mL)	Plasmid DNA (µg)	Serum-free Diluent (mL)	CalFectin™ Reagent (µL)
48-well plate	0.5	0.25	0.025	0.75
6-well plate	2	1	0.1	3
35 mm dish	2	1	0.1	3
60 mm dish	5	2.5	0.25	7.5
10 cm dish	10	4–6	0.5	12–18
T75 flask	10	4–6	0.5	12–18
150 mm Dish	20	10–20	1.25	30–60

Storage: CalFectin™ Reagent is stable for up to 24 months at +4 °C after receipt. Do not freeze.