SignaGen® Laboratories

CalFectin™ Mammalian DNA Transfection Reagent

A Protocol for Lentivirus Production in 293T Cells

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Introduction

CalFectin™ Mammalian DNA Transfection Reagent is a chemically optimized calcium phosphate-based transfection system that generates uniformly sized DNA–calcium co-precipitate complexes, enabling efficient endocytosis with reduced cytotoxicity relative to classical calcium phosphate methods. CalFectin™ is particularly well-suited for high-efficiency transient transfection of HEK293T cells, which is the basis for third-generation lentiviral vector production. This protocol describes the use of CalFectin™ for lentivirus packaging in 293T cells using a three-plasmid co-transfection system comprising: (1) the lentiviral transfer vector carrying the transgene of interest, (2) a packaging plasmid encoding the necessary viral structural and enzymatic proteins (e.g., psPAX2 or equivalent), and (3) a VSV-G envelope plasmid (e.g., pMD2.G) to pseudotype the resulting virions for broad tropism. This system produces replication-incompetent lentiviral particles suitable for stable gene delivery into dividing and non-dividing target cells.

> ■***Note:*** *Lentiviral vectors are classified as Biosafety Level 2 (BSL-2) agents. All work must be performed in accordance with institutional biosafety guidelines and applicable regulatory requirements. Consult your Institutional Biosafety Committee (IBC) before beginning lentiviral production experiments.*

Kit Contents

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

Shelf life: CalFectin™ Reagent is stable for up to 12 months at +4 °C after receipt. Do not freeze.

General Guidelines

- Always use serum-free tissue culture medium (e.g., DMEM with high glucose) to dilute both the DNA and CalFectin™ Reagent separately before mixing. Serum proteins inhibit DNA–calcium co-precipitate assembly, reducing particle formation and transfection efficiency. Do not use Opti-MEM for lentiviral production; its buffering composition and protein supplements interfere with calcium phosphate complex formation and are incompatible with this protocol.
- Pre-dilute the total plasmid DNA mixture into serum-free DMEM, then add CalFectin™ Reagent directly into the diluted DNA solution. Mixing the reagent and DNA in a single tube (rather than combining two pre-diluted solutions) is the recommended procedure for this format, as described in Step 2.
- A 3:1 CalFectin™ (µL) : DNA (µg) ratio is recommended for 293T lentiviral production. Divide the total DNA between the three plasmid components at the ratio specified for the packaging system used (see Step 2).
- High cell confluency (~90%) at the time of transfection is critical for lentivirus production. Densely packed 293T monolayers maximize virion budding and yield. This is distinct from standard transfection protocols, where 60–70% confluency is optimal.

Procedures for Lentivirus Production in 293T Cells

Step 1 — Cell Seeding

Seed 293T cells 18–24 hours prior to transfection so that the monolayer reaches approximately 90% confluency at the time of transfection. High cell density is specifically required for efficient lentivirus production; a densely packed monolayer maximizes virion budding efficiency. Supplement each dish with fresh complete culture medium (containing serum and antibiotics) 30–60 minutes before transfection.

> ■***Note:*** *293T cells should be maintained in active growth and used at low passage numbers (≤30 passages) to preserve high transfection competency and viral yield. Serum and antibiotics in the culture medium do not inhibit CalFectin™-mediated transfection, as the CalFectin™/DNA complex is pre-formed in serum-free medium prior to addition to the cells.*

Step 2 — Preparation of CalFectin™/DNA Complex

The following protocol is optimized for lentivirus production in a 15 cm dish using a total of 18 µg of plasmid DNA. Combine the three plasmid components at the following mass ratio: **9 µg transfer vector : 6 µg packaging plasmid (e.g., psPAX2) :**

3 μg VSV-G envelope plasmid (e.g., pMD2.G), for a total of 18 μg per 15 cm dish. This 3:2:1 ratio balances transfer vector packaging capacity against the stoichiometric requirements for efficient Gag-Pol and VSV-G expression.

1. In a sterile microcentrifuge tube, combine the total 18 μg plasmid DNA mixture (transfer vector + packaging plasmid + envelope plasmid at the ratio above) and dilute into 1000 μL of serum-free DMEM (High Glucose). Mix gently by pipetting up and down 3–4 times. Do not vortex.
2. Add 54 μL of CalFectin™ Reagent directly into the diluted DNA solution. Mix immediately by gentle pipetting up and down 3–4 times. Do not vortex after addition; vigorous mixing disrupts nascent DNA–calcium co-precipitate nucleation.
3. Incubate the CalFectin™/DNA complex for 10 –15 minutes at room temperature (18–25°C) to allow uniform DNA–calcium co-precipitate formation. Do not exceed 20 minutes.
4. Add the 1000 μL CalFectin™/DNA complex dropwise and evenly over the medium in the 15 cm dish. Distribute evenly by gently swirling the plate.

CRITICAL: Do not incubate the CalFectin™/DNA complex for more than 20 minutes. Prolonged incubation results in uncontrolled particle aggregation, leading to reduced cellular uptake, diminished transfection efficiency, and increased cytotoxicity. Do not vortex the mixture after combining DNA and CalFectin™ Reagent; vigorous mixing disrupts complex formation.

Step 3 — Medium Change and Lentivirus Harvest

Lentiviral particles are shed continuously into the culture supernatant following transfection. The medium change at 24 hours removes residual CalFectin™/DNA complex and cytotoxic byproducts, which would otherwise reduce cell viability and contaminate the viral preparation. Peak lentiviral titers are typically achieved between 48 and 72 hours post-transfection, corresponding to maximum transgene expression and virion budding from the producer cell monolayer.

1. At 24 hours post-transfection, carefully aspirate the medium and replace with fresh complete culture medium (18 mL per 15 cm dish) pre-warmed to 37°C. Avoid disturbing the producer cell monolayer.
2. At 48 hours post-transfection, collect the virus-containing supernatant. Centrifuge at 500 × g for 5 minutes at 4°C to pellet cell debris. Transfer the clarified supernatant to a fresh conical tube and store on ice or at –80°C, or proceed immediately to concentration.
3. At 72 hours post-transfection, collect a second harvest of virus-containing supernatant by the same procedure. The 72-hour fraction typically yields a comparable or higher titer than the 48-hour fraction and may be pooled with it.
4. For applications requiring high-titer stocks, concentrate the pooled supernatant by ultracentrifugation (25,000 × g, 2 hours, 4°C) or by PEG precipitation. Resuspend the viral pellet in an appropriate volume of sterile PBS or serum-free culture medium and store in single-use aliquots at –80°C.

■***Note:*** *Do not freeze-thaw viral stocks more than once; repeated freeze-thaw cycles significantly reduce infectious titer. Determine viral titer by an appropriate quantitative method — such as p24 antigen ELISA, qRT-PCR for viral genomic RNA, or a functional transduction assay with a target cell line — before use.*

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