SignaGen® Laboratories

CalFectin™ Mammalian DNA Transfection Reagent

A Protocol for Single-Component Recombinant Adenovirus Packaging in HEK293A Cells

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

Introduction

CalFectin™ is a chemically optimized calcium phosphate-based transfection reagent that generates uniformly sized DNA–calcium co-precipitate complexes, enabling efficient endocytosis with reduced cytotoxicity relative to classical calcium phosphate methods. This protocol describes **single-component recombinant adenoviral vector packaging** in **HEK293A cells** using a **6-well plate format**.

This **one-component system** uses a single, fully assembled recombinant adenoviral backbone plasmid carrying **deletions of the E1 and E3 regions**, in which the transgene cassette has already been incorporated into the adenoviral genome *in bacteria* (e.g., via *BJ5183* or *AdEasier E. coli* recombination). Deletion of **E1** renders the virus replication-incompetent in normal somatic cells; productive replication is rescued exclusively in HEK293 producer cells, which constitutively supply E1A and E1B in *trans*. Deletion of **E3**, which encodes immunomodulatory proteins non-essential for viral replication *in vitro*, creates additional cloning capacity (~3.1 kb) for larger transgene cassettes and reduces immunogenicity of the resulting vector *in vivo*. The plasmid is linearized with ***Pac*I** immediately before transfection to expose the ITRs and packaging signal (Ψ), initiating productive replication and encapsidation in HEK293 cells. This approach eliminates intracellular homologous recombination, reduces production time, and minimizes the risk of mixed or non-recombinant viral populations.

■ ***Note:*** *E1/E3-deleted replication-incompetent recombinant adenoviral vectors are classified as **BSL-2** agents. All work must be conducted in a certified Class II biological safety cabinet in compliance with institutional biosafety policies. Consult your IBC before initiating packaging.*

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

Shelf life: Stable for up to 24 months at +4 °C. Do not freeze.

General Guidelines

- Always dilute linearized plasmid DNA in **serum-free DMEM (High Glucose)** before adding CalFectin™. Serum proteins inhibit co-precipitate assembly. Do **not** use Opti-MEM; its buffering profile is incompatible with calcium phosphate complex formation.
- **Single-tube mixing format:** pre-dilute linearized DNA in serum-free DMEM, then add CalFectin™ directly to the DNA solution. Do not pre-dilute the reagent separately. Use a **3:1** CalFectin™ (µL) : DNA (µg) ratio.
- Target **60–70% confluency** at transfection. Active cell division is required for adenoviral genome replication; overly dense monolayers reduce uptake, while sub-confluent cultures dilute viral genomes through excessive post-transfection division.
- Use HEK293 cells at ≤**30 passages** for stable E1A/E1B complementation. **HEK293T cells are not recommended**: SV40 Large T-antigen expression can interfere with adenoviral replication and elevates the risk of generating replication-competent adenovirus (RCA).

Pre-Transfection: PacI Linearization of the Recombinant Adenoviral Backbone Plasmid

*Pac*I cleaves at two TTAATTAA sites flanking the adenoviral ITRs, releasing the full-length adenoviral genome from the prokaryotic plasmid backbone. Exposure of the ITRs is an absolute requirement for initiation of viral DNA replication by the adenoviral pol–pTP complex; uncut circular plasmid generates negligible viral yield.

1. Set up the *Pac*I digest: combine **1-3 µg recombinant adenoviral backbone plasmid**, **5 U *Pac*I per µg DNA**, and appropriate 10× buffer. Incubate at **37 °C for 2–4 h** (overnight for ≥10 µg).
2. Verify complete linearization by **0.8% agarose gel electrophoresis** alongside uncut plasmid. Complete digestion yields two bands: the linearized adenoviral genome (~26–30 kb for an E1/E3-deleted backbone) and the released prokaryotic backbone (~2.5–3.5 kb). **Do not proceed if uncut circular plasmid is visible.**
3. Proceed to transfection directly with the linearized adenoviral backbone plasmid mixture without purification **on the same day.**

■ ***CRITICAL:*** *Use linearized DNA on the same day as preparation. Storage at 4 °C or freeze–thaw causes ITR end-degradation and significantly reduces packaging efficiency. If same-day use is not possible, store as a single-use aliquot at –20 °C for ≤1 week.*

Transfection Setup — Per Well of a 6-Well Plate

Component	Amount	Notes
*Pac*I-linearized recombinant adenoviral backbone plasmid	1 µg	Fully assembled recombinant Ad genome with E1 and E3 deletions; transgene pre-inserted into E1 locus via bacterial recombination. ITRs and Ψ exposed by *Pac*I digestion.
CalFectin™ Reagent	3 µL	3:1 µL:µg ratio; added directly to diluted DNA (single-tube format)
Serum-free DMEM (High Glucose)	100 µL	Diluent for linearized DNA; do not substitute Opti-MEM

Step 1 — Cell Seeding

Seed HEK293 Acells into each well of a **6-well plate** with **2 mL complete DMEM** (10% FBS, 1% pen/strep) per well, **18–24 h prior to transfection**, targeting **70–80% confluency**. Replace with **2 mL fresh, pre-warmed (37 °C) complete DMEM** per well 30–60 minutes before complex addition.

■ ***Note:*** *HEK293 cells must be in active exponential growth (passage ≤30) for stable E1A/E1B complementation. Serum and antibiotics do not inhibit CalFectin™-mediated transfection, as the complex is pre-formed in serum-free medium before cell addition.*

Step 2 — Preparation of CalFectin™/DNA Complex (Per Well)

Prepare immediately before use. When transfecting ≥3 wells, prepare a master mix to reduce pipetting variability.

1. Add **1 µg *Pac*I-linearized adenoviral backbone plasmid** mixture to a sterile 1.5 mL tube. Dilute into **100 µL serum-free DMEM (High Glucose)**. Mix gently by pipetting 3–4×. Do *not* vortex.
2. Add 3 **µL CalFectin™ Reagent** directly into the diluted DNA solution. Mix immediately by gentle pipetting 3–4×. Do *not* vortex — vigorous mixing disrupts nascent DNA–calcium co-precipitate nucleation.
3. Incubate at **room temperature (18–25 °C) for exactly 10 minutes**. Do *not* exceed 20 minutes.
4. Add the above **transfection mix dropwise and evenly** over the medium in a well of 6-well plate. Distribute by gently tilting the plate. Return the plate to **37 °C / 5%CO_2** immediately.

■ ***CRITICAL:*** *Do not incubate the CalFectin™/DNA complex for more than 20 minutes. Over-incubation causes uncontrolled aggregation, reducing uptake, efficiency, and cell viability. Never vortex after combining linearized DNA and CalFectin™ Reagent.*

Step 3 — Medium Change (24 h Post-Transfection)

At **24 h post-transfection**, aspirate medium and replace each well with **2 mL fresh, pre-warmed complete DMEM** to remove residual CalFectin™/DNA complexes and cytotoxic by-products, preserving cell viability through the replication phase.

■ ***Note:*** *Because bacterial recombination has already assembled the full-length adenoviral genome prior to transfection, no intracellular homologous recombination is required. Viral replication initiates directly from the linearized ITR termini upon nuclear import, accelerating first CPE onset to typically **7–10 days post-transfection** compared with 10–14 days for two-plasmid systems.*

Step 4 — Monitoring and Primary Harvest (Days 7–10 Post-Transfection)

Adenoviral CPE — cell rounding, refractility, detachment, and monolayer lysis — typically appears **7–10 days post-transfection** and progresses as plaques expand.

1. Inspect each well daily by phase-contrast microscopy from **day 5**. Replace 1 mL medium every 3–4 days with pre-warmed complete DMEM to maintain viability in non-lysed regions.
2. When ≥**80% CPE** is observed, scrape remaining adherent cells and transfer the complete suspension (cells + supernatant) to a **15 mL conical tube**. This is the **P0 crude lysate**.
3. Lyse by **three freeze–thaw cycles** (dry ice/ethanol snap-freeze 5 min, thaw 37 °C with vortexing). Clarify at **500 × g, 5 min, 4 °C**. Collect the clarified supernatant as the P0 viral stock.
4. Confirm transgene integrity by **restriction digest or PCR on the P0 lysate** prior to amplification, to verify recombinant genome structure and exclude deletions or rearrangements arising during bacterial recombination or viral replication.

■ ***CRITICAL:*** *P0 crude lysate titer is typically 10^6–10^7 IFU/mL and is **not suitable for direct experimental use**. Proceed to serial amplification in HEK293 cells (P1 → P2 → P3) to reach ≥10^{10} IFU/mL before purification and titering. Store P0 at –80 °C until amplification.*

Storage

- **CalFectin™ Reagent:** Stable for up to 24 months at +4 °C. Do not freeze.
- ***Pac*I-linearized adenoviral genome:** Use on the day of preparation. If necessary, store as a single-use aliquot at –20 °C for ≤1 week. ITR end-degradation occurs at 4 °C.
- **P0 crude lysate and amplified stocks (P1–P3):** Store as single-use aliquots at –80 °C. Repeated freeze–thaw progressively reduces infectious titer.

SignaGen Laboratories | 5260 Westview Dr, Suite 100, Frederick MD 21703 | Tel: 301-330-5966 | Toll Free: 1-866-918-6812 | info@signagen.com | www.signagen.com