SKU # SL100668 Store at 4 °C

LipoD293™ In Vitro DNA Transfection Reagent (Ver. II)

----- A Protocol for Adenovirus Packaging in HEK293A Cells

☐ 100 µL
☐ 500 µL
☐ 1000 µL

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This product is for laboratory research ONLY and not for diagnostic use

Introduction:

Leveraging our innovative proprietary lipid conjugation technology, LipoD293™ (Ver. II) has been specifically engineered and formulated with proprietary transfection enhancers to achieve highly efficient DNA delivery into HEK293 cells and a broad range of mammalian cell types. As an enhanced liposome-based DNA transfection reagent, LipoD293™ (Ver. II) consistently provides exceptionally high transfection efficiency in HEK293-derived cells and many other mammalian cell lines while maintaining minimal cytotoxicity and excellent cell viability.

This protocol describes the production of recombinant adenoviral vectors (rAd) in HEK293A cells using a single-component, self-contained adenoviral backbone plasmid system (e.g., pAd/PL-DEST™ or equivalent) in a 6-well plate format. In this approach, the transgene of interest is pre-cloned into the adenoviral backbone plasmid, which carries all viral genes required for replication and packaging in trans, eliminating the need for a separate helper virus.

Linearization of the plasmid prior to transfection is required to expose the inverted terminal repeats (ITRs) and facilitate rescue of infectious adenoviral particles. HEK293A cells, which stably express the adenoviral E1A and E1B gene products, provide the E1 functions necessary for productive replication of E1-deleted recombinant adenoviral vectors.

This system yields replication-incompetent, E1/E3-deleted recombinant adenoviral particles with an increased transgene packaging capacity of up to ~8 kb, suitable for efficient transduction of a broad range of dividing and non-dividing mammalian cell types. All transfection conditions specified for the 6-well plate format are directly scalable to larger culture vessel formats in proportion to the tissue culture surface area.

Note: *Recombinant adenoviral vectors are generally classified as BSL-1 agents. However, vectors carrying potentially oncogenic transgenes or immunomodulatory sequences may require BSL-2 containment. Consult your Institutional Biosafety Committee (IBC) prior to initiating production.*

General Guidelines:

- Always dilute LipoD293™ Reagent and plasmid DNA in serum- and antibiotic-free DMEM (High Glucose) prior to complexation. Serum proteins interfere with lipoplex assembly and reduce transfection efficiency.
- Do not use Opti-MEM as the diluent. Its buffering composition is incompatible with LipoD293™-DNA complex formation and significantly reduces transfection efficiency.
- Add LipoD293™ Reagent directly into the diluted DNA solution (single-tube format). Use a 3:1 LipoD293™ (µL) : DNA (µg) ratio as the initial condition.
- The adenoviral backbone plasmid must be linearized with an appropriate restriction endonuclease (e.g., PacI) prior to transfection. Confirm complete linearization by agarose gel electrophoresis prior to use.
- Use HEK293A cells at ≤30 passages to preserve stable E1A/E1B expression and optimal permissivity for adenoviral replication.
- Target 70–80% confluency at the time of transfection. Subconfluent monolayers reduce transfection uptake; overly dense cultures compromise viral amplification efficiency.

Step 1 — Plasmid Linearization

Digest the adenoviral backbone plasmid to completion using an appropriate restriction endonuclease that cuts within the plasmid backbone but outside the adenoviral ITRs (e.g., PacI for pAd/PL-DEST™ -based systems). Use 5–10 units of enzyme per µg of plasmid DNA and incubate under the manufacturer's recommended conditions for a minimum of 2 hours to ensure complete digestion.

Confirm complete linearization by agarose gel electrophoresis. The linearized adenoviral backbone plasmid mixture can be used directly for transfection without requiring further purification.

Note: *Incomplete linearization results in significantly reduced viral rescue efficiency. Circular plasmid is unable to expose the ITRs required for initiation of adenoviral DNA replication. Do not proceed to transfection unless complete linearization is confirmed by gel electrophoresis.*

Step 2 — Cell Seeding

Seed HEK293A cells in a 6-well plate at a density sufficient to achieve 70–80% confluency 18–24 hours after seeding. Aspirate and add 2.0 mL of fresh, pre-warmed (37 ºC) complete medium 30–60 minutes before complex addition to stabilize pH and replenish nutrients.

Note: *Verify monolayer morphology by phase-contrast microscopy prior to transfection. Unhealthy or over-passaged HEK293A cells are the most common cause of failed viral rescue. Serum and antibiotics in the culture medium do not inhibit LipoD293™-mediated transfection, as the complex is pre-formed in serum-free medium prior to addition to cells.*

Step 3 — Preparation of LipoD293™-DNA Complex

For each well of a 6-well plate:

1. In a sterile 1.5 mL microcentrifuge tube, add **1.0 µg** of linearized adenoviral backbone plasmid mixture to 1**00 µL** of serum- and antibiotic-free DMEM (High Glucose). Mix gently by pipetting 3–4 times.
2. Add 3.0 **µL** of LipoD293™ Reagent (3:1 µL:µg ratio) directly into the diluted DNA solution. Mix immediately by gentle pipetting 3–4 times.
3. Incubate at room temperature (18–25 ºC) for exactly 10-20 minutes to allow uniform lipoplex formation. Do not exceed 20 minutes.
4. Add the entire LipoD293™/DNA complex dropwise and uniformly over the HEK293A cell monolayer in each well.

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Ensure homogeneous distribution by gently rocking the plate in a cross-wise pattern immediately after addition. Return the plate to the 37 ºC / 5% CO_2 incubator immediately.

⚠ ***CRITICAL:*** *Do not incubate the LipoD293™/DNA complex for more than 20 minutes prior to addition to cells. Over-incubation causes uncontrolled lipoplex aggregation, reducing cellular uptake, transfection efficiency, and cell viability.*

Step 4 — Post-Transfection Monitoring

Change medium 24 hours post-transfection. Inspect the transfected HEK293A monolayer daily by phase-contrast microscopy beginning at 48 hours post-transfection. Monitor for the appearance of cytopathic effect (CPE), characterized by cell rounding, clustering, refractility, and progressive detachment from the culture surface. CPE typically appears within 7–14 days post-transfection and indicates productive adenoviral replication and particle release.

■ ***Note:*** *The onset and progression of CPE is highly dependent on transfection efficiency, plasmid quality, and cell passage number. If no CPE is observed by day 14, a blind passage should be performed: collect the cells and supernatant, lyse by three freeze–thaw cycles, and use the clarified lysate to infect a fresh HEK293A monolayer in a 6-well plate.*

Step 5 — Primary Virus Harvest (P0)

Once CPE is observed in ≥80% of the monolayer, proceed with primary virus harvest (passage 0, P0):

1. Dislodge cells by scraping into the existing medium. Transfer the entire cell suspension from all wells into a 15 mL conical tube.
2. Pellet cells by centrifugation at 500 x g for 5 minutes at 4 ºC. Discard the supernatant.
3. Resuspend the combined cell pellet in **1.0 mL** of sterile PBS (pH 7.4).
4. Lyse by three freeze–thaw cycles: snap-freeze in a dry ice/ethanol bath for 5 minutes, then thaw at 37 ºC with vortexing. Repeat for a total of three cycles.
5. Clarify the crude lysate by centrifugation at 2,000 x g for 10 minutes at 4 ºC. Collect the clarified supernatant (crude viral lysate, P0) into a fresh 1.5 mL microcentrifuge tube.

⚠ ***CRITICAL:*** *Do not exceed three freeze–thaw cycles. Repeated freeze–thaw causes progressive loss of viral capsid integrity and significantly reduces infectious titer. Aliquot the P0 crude lysate as single-use volumes and store at −80 ºC until use.*

Step 6 — Viral Amplification (P1 and P2)

The P0 crude lysate obtained from a 6-well plate typically contains insufficient titer for direct experimental use and must be amplified through sequential passaging in HEK293A cells.

P1 Amplification (6-well plate → 10 cm dish): Infect a 70–80% confluent monolayer of HEK293A cells in a 10 cm dish with the entire P0 crude lysate (1.0 mL). Monitor daily for CPE by phase-contrast microscopy. When CPE is observed in ≥80% of the monolayer, harvest by scraping into the existing medium and transfer to a 15 mL conical tube. Pellet at 500 x g for 5 minutes at 4 ºC, discard the supernatant, and resuspend the cell pellet in **5.0 mL** of sterile PBS. Lyse by three freeze–thaw cycles and clarify at 2,000 x g for 10 minutes at 4 ºC. This constitutes the P1 lysate.

P2 Amplification (10 cm dish →5 x 15 cm dish): Infect a 70–80% confluent monolayer of HEK293A cells in five (5) 15 cm dish each with 1.0 mL P1 lysate. Harvest as described above when CPE reaches ≥80%. Resuspend the cell pellet harvested from five 15 cm plates into **10.0 mL** of sterile PBS. Lyse by three freeze–thaw cycles and clarify at 2,000 x g for 10 minutes at 4 ºC. This constitutes the P2 lysate, which is suitable for large-scale purification.

■ ***Note:*** *For high-titer, research-grade adenoviral preparations, proceed to CsCl density gradient ultracentrifugation or column-based purification (e.g., iodixanol gradient or commercial adenovirus purification kit) following P2 amplification. Crude lysates are not suitable for in vivo applications.*

Step 7 — Assessment of Viral Titer

Determine the infectious titer of the amplified adenoviral preparation using one or more of the following methods prior to experimental use:

1. **Plaque Assay:** Infect HEK293A monolayers in 6-well plates with serial dilutions of the viral preparation under a semi-solid agarose or carboxymethylcellulose (CMC) overlay. Count plaques at 7–10 days post-infection. Reports titer as plaque-forming units per mL (PFU/mL).
2. **$TCID_{50}$ Assay:** Infect HEK293A cells in 96-well plates with serial 10-fold dilutions. Score CPE at day 10–14. Calculate titer using the Reed–Muench method; reports as the 50% tissue culture infectious dose per mL ($TCID_{50}$/mL).
3. **Optical Particle Quantification (OD_{260}):** Measure absorbance of purified viral preparation at 260 nm. One OD_{260} unit ≈ 1×10^{12} viral particles (VP)/mL. Reports physical particle titer; does not distinguish infectious from non-infectious particles.
4. **qPCR:** Quantify adenoviral genome copies using primers targeting a conserved adenoviral sequence (e.g., hexon gene). Reports genome copies per mL (GC/mL).

Storage: LipoD293™ In Vitro DNA Transfection Reagent Ver. II is stable for 24 months at +4 ºC. This item shipped at ambient temperature

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