SKU # SL100489 Store at 4 °C

GenJet™ In Vitro DNA Transfection Reagent (Version II)

----- A Protocol for AAV Packaging in HEK293T Cells

- ☐ 100 µL
- ☐ 500 µL
- ☐ 1000 µL

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

Introduction:

GenJet™ In Vitro DNA Transfection Reagent (Ver. II) is an advanced-generation formulation of GenJet™ In Vitro DNA Transfection Reagent incorporating an optimized chemical composition. The reformulated chemistry incorporates an increased density of DNA-condensing moieties relative to the original GenJet™ formulation, resulting in more compact and stable lipoplex formation. This protocol describes a fully helper-free triple-plasmid system for recombinant AAV (rAAV) production in HEK293T cells, eliminating the requirement for live adenovirus or herpesvirus co-infection.

The three-plasmid components are: (1) an ITR-flanked AAV transfer vector carrying the transgene of interest; (2) a serotype-specific Rep/Cap plasmid providing the AAV replication and capsid proteins; and (3) a cis-deleted adenoviral helper plasmid (e.g., pHelper or pAdDeltaF6) supplying E2A, E4orf6, and VA RNA functions without producing infectious adenovirus. This system yields replication-incompetent rAAV particles suitable for stable transduction of dividing and non-dividing target cells.

■ ***Note:*** *Recombinant AAV vectors are generally classified as BSL-1 agents; however, vectors carrying potentially oncogenic transgenes or pseudotyped with certain capsid serotypes may require BSL-2 containment. Consult your Institutional Biosafety Committee (IBC) prior to initiating production.*

General Guidelines:

- Always dilute plasmid DNA and GenJet™ Reagent Ver. II in serum-free DMEM with high glucose prior to mixing. Serum proteins inhibit GenJet™-DNA transfection complex formation. Do not use Opti-MEM; its buffering composition is incompatible with transfection complex formation.
- Add GenJet™ Reagent Ver. II directly into the diluted DNA solution (single-tube format); do not pre-dilute the reagent separately. Use a 3:1 GenJet™ (µL) : DNA (µg) ratio.
- Target 70–80% confluency at transfection. AAV assembly is intranuclear; sub-confluent cells maintain sufficient mitotic activity and nuclear capacity for productive replication.
- Use HEK293T cells at ≤30 passages to preserve transfection competency and stable SV40 Large T-antigen expression, which drives episomal plasmid amplification.

Plasmid Composition (Per 15 cm Dish — Total 18 µg):

Plasmid	Amount	Function
ITR-flanked transfer vector	5 µg	Transgene cassette flanked by AAV2 ITRs
pAAV-RC (serotype-specific Rep/Cap)	5 µg	AAV Rep and Cap (capsid) proteins
pHelper / pAdDeltaF6 (adenoviral helper, replication-incompetent)	8 µg	E2A, E4orf6, VA RNA — helper functions without producing infectious adenovirus

The 5:5:8 mass ratio (transfer vector : Rep/Cap : helper) reflects the stoichiometric requirements of the helper-free system: Rep/Cap are needed equimolarly relative to the ITR template, while adenoviral helper functions are rate-limiting and benefit from a twofold molar excess. Deviation from this ratio reduces encapsidation efficiency and viral yield.

AAV Packaging Procedures:

Step 1 — Cell Seeding

Seed HEK293T cells in a 15 cm dish containing 20 mL of complete DMEM with high glucose (10% FBS, 1% penicillin/streptomycin) 18–24 hours prior to transfection, targeting 70–80% confluency at the time of transfection. Add 20 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 cells are the most common cause of low viral yield. Serum and antibiotics in the culture medium do not inhibit GenJet™ mediated transfection, as the complex is pre-formed in serum free medium before addition to cells.*

Step 2 — Preparation of GenJet™-DNA Complex

1. In a sterile 1.5 mL microcentrifuge tube, combine the 18 µg total plasmid DNA (5 µg transfer vector + 5 µg Rep/Cap + 8 µg pHelper) and dilute into 1,000 µL serum-free DMEM (High Glucose). Mix gently by pipetting 3–4 times.
2. Add 54 µL (GenJet™ Reagent Ver. II 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 ~10 minutes to allow stable formation of the transfection complex. Do not exceed 20 minutes.
4. Add the entire GenJet™–DNA transfection complex dropwise and uniformly over the HEK293T cell monolayer in the 15 cm dish. Ensure homogeneous distribution by gently rocking the plate in a cross-wise pattern immediately after addition. Return the dish to the 37 ºC / 5% CO_2 incubator immediately.

⚠ ***CRITICAL:*** *Do not incubate the GenJet™-DNA complex for longer than 20 minutes. Over-incubation causes uncontrolled particle aggregation, reducing cellular uptake, transfection efficiency, and cell viability.*

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

At 24 hours post-transfection, carefully aspirate the medium and replace with 20 mL of fresh, pre-warmed (37 ºC) complete DMEM. This step removes residual GenJet™/DNA complexes and cytotoxic by-products that accumulate during the initial transfection window, preserving cell viability through the peak

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production phase.

*■ **Note:** Unlike lentiviral vectors, which are constitutively shed into the supernatant, the majority of rAAV particles remain cell-associated and are released only upon lysis. Supernatant-based harvest captures only a minor fraction of total yield and is not the primary collection strategy in this protocol.*

Step 4 — AAV Harvest by Cell Lysis (72 h Post-Transfection)

1. Peak intracellular AAV titers are typically achieved at 72 hours post-transfection, corresponding to maximal Rep-mediated genome replication, capsid assembly, and DNA packaging.
2. Inspect producer cells by phase-contrast microscopy. Cytopathic changes (rounding, granularity, partial detachment) are expected and indicate productive replication. Proceed with harvest regardless of the degree of cytopathic effect.
3. Dislodge adherent cells by scraping into the existing medium. Transfer the suspension to a 50 mL conical tube. Pellet at 300 x g, 5 min, 4 ºC. Discard supernatant (or pool with cell pellet if maximal recovery is required).
4. Resuspend the cell pellet in 1 mL of lysis buffer per 15 cm dish (150 mM NaCl, 50 mM Tris-HCl, pH 8.5). Lyse by three freeze–thaw cycles: snap-freeze in dry ice/ethanol (5 min), then thaw at 37 ºC with vortexing. Repeat for a total of three cycles.
5. Add Benzonase® nuclease to 50 U/mL and $MgCl_2$ to 1 mM. Incubate at 37 ºC for 30 minutes with gentle agitation. This degrades residual plasmid DNA and unpackaged AAV genomes without affecting encapsidated particles, substantially improving preparation purity.
6. Clarify the lysate by centrifugation at 4,000 x g, 20 min, 4 ºC. Collect the clarified supernatant (crude viral lysate) into a fresh tube. Proceed to downstream purification immediately, or store at −80 ºC.

*⚠ **CRITICAL:** Do not exceed three freeze–thaw cycles for viral stocks; repeated cycling significantly reduces infectious titer. Aliquot purified vector as single-use volumes and store at −80 ºC. Avoid repeated freeze–thaw of working aliquots.*

Storage: GenJet™ Reagent Ver. II is stable for 24 months at +4 °C. This item shipped at ambient temperature