SKU # SL100489 Store at 4 °C

GenJet™ In Vitro DNA Transfection Reagent (Version II)

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

- ☐ 100 µL
- ☐ 500 µL
- ☐ 1000 µL

SignaGen® Laboratories
5260 Westview Dr, Suite 100
Frederick MD 21703
FAX. 301-560-4919
TEL. 301-330-5966
Toll Free. 1-(866)-918-6812
Email: info@signagen.com
Web: www.signagen.com

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. GenJet™ Reagent Ver. II 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 GenJet™ Reagent Ver. II 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.*

General Guidelines:

- Always use serum-free tissue culture medium (e.g., DMEM with high glucose) to dilute both the DNA and GenJet™ Reagent Ver. II before mixing. Serum proteins inhibit GenJet™ -DNA complex formation and transfection efficiency. Do not use Opti-MEM for lentiviral production; its buffering composition and protein supplements interfere with transfection complex formation and are incompatible with this protocol.
- Pre-dilute the total plasmid DNA mixture into serum-free DMEM, then add GenJet™ Reagent Ver. II 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 GenJet™ (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. The following protocol is optimized for lentivirus production in a 15 cm dish with 20 mL of complete DMEM with high glucose supplemented with 10% FBS and antibiotics.

*■ **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 GenJet™-mediated transfection, as the GenJet™/DNA complex is pre-formed in serum-free medium prior to addition to the cells.*

Step 2 — Preparation of GenJet™-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 GenJet™ Reagent Ver. II directly into the diluted DNA solution. Mix immediately by gentle pipetting up and down 3–4 times. Do not vortex after addition.
3. Incubate the GenJet™-DNA mix for ~10 minutes at room temperature (18–25 ºC) to allow stable transfection complex formation. 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 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 GenJet™ Reagent; Vigorous mixing may disrupt 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 GenJet™/DNA complex and cytotoxic byproducts, which would otherwise reduce cell viability and

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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 (20 mL per 15 cm dish) pre-warmed to 37 ºC. Avoid disturbing the producer cell monolayer.
2. At 48 hours post-transfection, carefully aspirate and collect the virus-containing supernatant. Replenish the producer cell monolayer with an equivalent volume of fresh, pre-warmed (37 ºC) complete DMEM (High Glucose) supplemented with 10% FBS and 1% penicillin/streptomycin to support continued viral production for the 72-hour harvest. Clarify the collected supernatant by centrifugation at 500 × g for 5 minutes at 4 ºC to pellet cellular debris. Transfer the clarified supernatant to a fresh 50 mL conical tube and either store at −80 ºC or proceed immediately to downstream concentration and purification.
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 x g, 2 hours, 4 ºC) 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 ºC.

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