

Lentiviral Particle Transduction Protocol

Version: 3.1

Overview

The LipExoGen Lentiviral Expression System™ uses a third-generation, replication-incompetent, VSV-G-pseudotyped HIV-1-based vector for efficient transduction of dividing and non-dividing mammalian cells. Particles are purified by PEG precipitation and sucrose gradient centrifugation, suspended in serum-free RPMI 1640 medium, and provided at high functional titer for consistent performance.

General Handling and Storage

- Upon receipt, store lentiviral vials at **-80°C**.
 - Thaw vials only immediately before use. Avoid multiple freeze-thaw cycles to preserve transduction efficiency.
 - After first thaw, aliquot any unused virus and return immediately to -80°C.
 - Typical vial volume: 380–500 µL.
 - Fluorescent reporters: RFP (mCherry) excitation/emission peaks at 587/610 nm. The GFP used in our reporters is standard EGFP (ex/em: 488/509 nm).
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Transduction Protocol

Adherent Cells

1. Plate $1\text{--}3 \times 10^5$ cells per well in a 24- or 12-well plate. Incubate overnight at 37°C, 5% CO₂.
2. Replace medium with fresh medium containing 6–9 µg/mL polybrene. Mix gently.
3. Add freshly thawed lentivirus at the recommended volume (see below) and mix by gentle swirling.
4. Incubate cells at 37°C, 5% CO₂ for 48–72 hours.
5. If toxicity occurs, replace medium after 16 hours.
6. Passage cells 48–72 hours post-infection before drug selection or gene expression analysis.

Puromycin Selection:

- Begin puromycin selection at 1 µg/mL 72 hours after transduction.
 - Increase to 2 µg/mL only after confirming robust cell recovery.
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Floating Cells

1. On the day of transduction, pool and resuspend cells at $3\text{--}5 \times 10^5$ cells per well in a 24-well plate.
2. Add 6–9 µg/mL polybrene, mix by resuspension.
3. Add freshly thawed lentivirus, mix gently.
4. Centrifuge plate at 2500 rpm, 30°C for 2 hours (spinoculation).
5. After centrifugation, incubate at 37°C, 5% CO₂ for 2–4 hours.
6. Transfer entire culture to a 25 cm² flask and continue culture for 48–72 hours.
7. If toxicity occurs, pellet cells after 16 hours, resuspend in fresh medium, and continue culture.

Puromycin Selection:

Same as adherent cells: start at 1 µg/mL 72 hours after transduction, and increase to 2 µg/mL following recovery.

Special Protocol for Difficult Cells

(Primary cells, floating cells, frozen-thawed cells)

If standard methods are insufficient:

1. **Plate Coating:**
 - Coat a 12-well plate with 1 mL RPMI 1640 + 60 µg/mL polybrene. Incubate 1 hour at 37°C.
2. **Cell Preparation:**
 - Suspend $0.5\text{--}1 \times 10^6$ cells in 500 µL fresh medium.

- Add 9 µg/mL polybrene, mix well.
- 3. Infection:**
 - Add 50–100 µL freshly thawed lentivirus. Mix thoroughly.
- 4. Pre-Incubation:**
 - Incubate virus-cell mix for 30–60 minutes at 37°C.
- 5. Transfer and Spin:**
 - Remove polybrene from coated well, add 1 mL fresh medium, and transfer virus-cell mix.
 - Centrifuge at 2200–2500 rpm, 30°C, 2 hours.
- 6. Culture:**
 - Incubate an additional 2–4 hours post-spin.
 - Transfer to a 25 cm² flask and culture for 3 days before selection.

Determining the Optimal Amount of Virus

- Single reporters (EGFP/mCherry only): 20–50 µL per $1-5 \times 10^5$ cells.
- Luciferase or dual reporters (e.g., GFP+RFP): 50–100 µL per $3-5 \times 10^5$ cells.
- Primary or difficult cells: Use higher cell densities ($0.5-1 \times 10^6$ cells) and start at 50–100 µL virus.
- Jurkat cells and T cells: Be cautious with puromycin; begin at 1 µg/mL only.

Important Tips:

- Titer (TU) is determined in-house using HEK293FT cells.
- Efficiency can vary based on insert size, number of repeats, promoter strength, cell type, and other factors.
- Always titrate virus amounts when establishing a new system.
- For multiple constructs, sequential transduction is generally preferred. Co-transduction can work for smaller payloads (e.g., EGFP, mCherry, blasticidin), but efficiency may drop with larger elements like firefly luciferase or puromycin. If needed, split delivery into separate steps.
- We recommend performing a drug titration beforehand to assess cell sensitivity, as recommended doses may not suit all applications.

Antibiotic Selection Guidance

- Start puromycin at 1 µg/mL, 72 hours after transduction. Increase to 2 µg/mL only after visible recovery.
- Typical selection concentrations for other markers: Blasticidin, 10 µg/mL; Hygromycin, 250 µg/mL; Zeocin, 500 µg/mL.

Frequently Asked Questions (FAQs)

- 1. What titer is the virus? Can I use it in primary or freeze-thawed cells?** Each vial contains at least 5×10^6 TU (GFP/RFP) or 2×10^6 TU (Luciferase), determined by transduction in HEK293FT cells. PEG purification and sucrose gradient concentration make them suitable for primary and freeze-thawed cells.
- 2. Can I use this product to generate stable cell lines?** Yes. Each construct includes puromycin or blasticidin resistance for straightforward stable line generation.
- 3. What MOI should I use?** We do not recommend a fixed MOI. It depends on your cell type and assay. Titrate virus amount for your specific conditions following this protocol.
- 4. Can I infect stem cells with this product?** Yes. Many customers have successfully transduced stem cells using our lentiviral reporters.
- 5. My cells all died after puromycin selection. What should I do?** Start with 0.5–1 µg/mL puromycin and only increase after confirming cell recovery. Full resistance typically takes at least 72 hours post-transduction to develop.
- 6. How much virus is in one vial?** Each vial contains approximately 380–500 µL of concentrated lentivirus. Titer is printed on the vial label.
- 7. I'm not seeing fluorescence, but my cells express the TF of interest.** Expression isn't enough. The TF must be active, nuclear-localized, and often part of a protein complex. These factors vary by cell type. Just because the TF is present doesn't mean it's doing anything. If you're unsure, controls and functional validation are key.
- 8. I see weak signal. Is it real?** Maybe. Weak reporters may still be real—but background is always a factor. That's what the negative control is for. If your signal isn't clearly distinguishable, don't guess—compare.