

Abstract

Lentiviral vectors are quickly becoming a vector of choice for many in vivo gene delivery and RNA interference applications. However, lentiviral vector production remains a time consuming and relatively low yield process. In this study we compared the efficiency of lentiviral production using a standard calcium phosphate protocol versus a protocol using a high efficiency, low toxicity, lipopolyplex formulation (*TransIT*[®]-LT1 Reagent). The goal of this work was to determine the overall titer and time course of lentivirus formation using a three plasmid co-transfection strategy.

As expected, the results of these experiments demonstrated that both methodologies could be used to generate infectious lentiviral vectors. However, the lipopolyplex mediated transfection protocol (*TransIT*-LT1 Reagent) facilitated higher lentiviral titers in a significantly shorter time course. In addition to the more rapid timecourse of lentiviral production, higher overall titers were obtained at 24 and 48 hours post transfection and there was an added benefit of not needing to change the medium during the viral grow-up phase when using the low toxicity lipopolyplex formulation.

General Methods

Lentiviruses expressing EGFP were produced by transfecting three lentiviral plasmids (EGFP-expressing lentiviral vector, gag-pol expression plasmid, and a VSV-G envelope plasmid) into HEK 293FT cells in 6-well tissue culture plates using either calcium phosphate co-precipitation or lipopolyplex-mediated transfection with the *TransIT*-LT1 Transfection Reagent (Mirus Bio). Figure 1 illustrates the two transfection protocols.

Standard plasmid cocktails used for calcium phosphate transfection contained the following amounts of each of the three plasmids:

EGFP-lentiviral plasmid -	5 µg
gag-pol plasmid -	3.75 µg
VSV G envelope plasmid -	1.5 µg
	10.25 µg total

Transfections using *TransIT*-LT1 Reagent used half the amount of each plasmid listed to be more in line with the manufacturer's recommendations.

Lentivirus titers were assayed by harvesting culture supernatants at various timepoints post-transfection, serially diluting the supernatants, and infecting fresh HEK 293FT cells with the various dilutions. Four days post-infection, the number of EGFP expressing cells was determined, and used to back-calculate the number of infectious lentivirus particles per ml of original culture supernatant.

Results and Discussion

Figure 1. Transfection Protocols

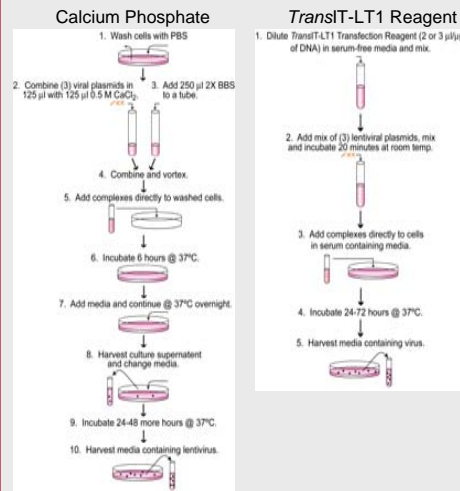
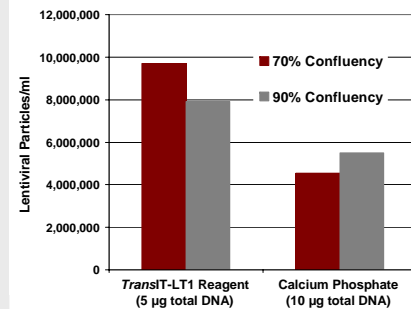
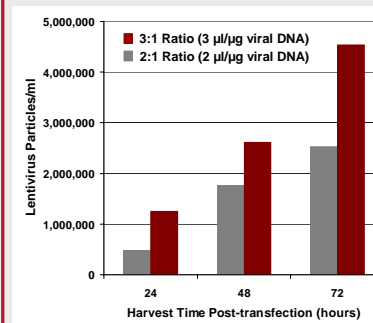


Figure 2. Transfection at 70% Confluency Is Suitable for Both Transfection Methods



Our previously optimized calcium phosphate protocol recommends transfecting cells at 90% confluency; the *TransIT*-LT1 Reagent protocol recommends transfecting cells at 50%-80% confluency. To test the effect of cell confluency on lentivirus yield, we transfected cells at both 70% and 90% confluency, using both methods. Twenty-four hours post-transfection culture supernatants were harvested and assayed for infectious lentivirus. Virus yield was about 20% greater when transfecting cells at 70% confluency using the *TransIT*-LT1 Reagent. The difference in confluency had a minimal effect on lentivirus yield when using the calcium phosphate method.

Figure 3. A 3:1 Ratio of *TransIT*-LT1 Reagent: Viral DNA is Optimal



To determine the optimal ratio of *TransIT*-LT1 Reagent to lentiviral plasmid DNA, two different ratios were tested. The first transfection contained 10 µl of *TransIT*-LT1 Reagent per 5 µg of total lentiviral plasmid DNA (2:1 ratio). The second transfection used 15 µl of *TransIT*-LT1 Reagent per 5 µg of total lentiviral plasmid DNA (3:1 ratio). Both transfection complexes were formed in parallel and transfected into the HEK 293FT cells. At 24, 48, and 72 hours post-transfection, the culture supernatants were harvested and assayed for lentivirus. As shown, the 3:1 ratio of *TransIT*-LT1 Reagent to lentiviral plasmid DNA produced significantly higher lentiviral titers than the cells transfected using the 2:1 ratio of *TransIT*-LT1 Reagent to lentiviral plasmids.

Optimal Conditions

Optimal lentiviral plasmid transfection conditions using *TransIT*-LT1 Reagent:

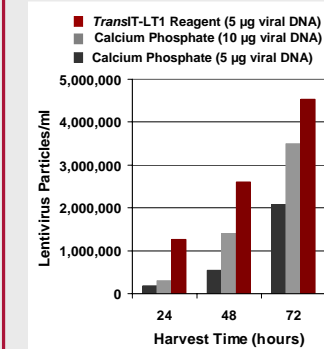
- 70% confluent HEK 293FT cells
- 5 µg of total viral plasmid DNA transfected/well
- 15 µl of *TransIT*-LT1 Reagent/5 µg plasmid DNA
- Harvest culture supernatants 72 hours post-transfection for maximal viral yield.

Conclusions

Calcium phosphate and *TransIT*-LT1 Reagent transfections produce high lentiviral titers, but use of the *TransIT*-LT1 Reagent offers several benefits over calcium phosphate co-precipitation.

1. 30-300% increase in lentiviral titers (depending on harvest time) when using the *TransIT*-LT1 Reagent.
2. *TransIT*-LT1 Reagent transfections require 50% less viral plasmid DNA to produce increased lentivirus yields.
3. The *TransIT*-LT1 Reagent protocol has fewer steps than the calcium phosphate protocol including no wash step, no media addition after complex addition, and no media change 24 hours post-transfection.

Figure 4. Increased Lentivirus Production When Using *TransIT*-LT1 Reagent to Transfect the Lentiviral Plasmids



HEK 293FT cells were transfected using either *TransIT*-LT1 Reagent (15 µl / 5 µg lentiviral plasmid DNA), or calcium phosphate co-precipitation (10 µg or 5 µg of lentiviral plasmid DNA). As shown, the *TransIT*-LT1 Reagent transfections yielded significantly higher lentiviral titers at all harvest times post-transfection compared to calcium phosphate transfections performed with equal or 2X more lentiviral plasmid DNA.