Highly Efficient Delivery of Viral RNA Genomes Using a Lipid-Based Transfection Reagent Compared to Electroporation

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Abstract

The production of infectious virus by the transfection of viral RNA genomes is an integral tool in dissecting the life cycles and pathogenesis of RNA viruses. Historically, electroporation has been the method of choice for the transfection of viral RNA genomes and mini-replicons, but it suffers from many limitations including high cell death, serum-free transfection conditions, and the requirements for large numbers of cells and large amounts of transfected RNA. To optimize and facilitate the introduction of viral RNAs into mammalian cells, we compared lipid-based transfection reagents and electroporation for their ability to deliver hepatitis C virus (HCV) and yellow fever virus replicons to HuH7 cells. We have identified a lipid-based transfection reagent that enables highly efficient delivery of the viral replicons to HuH7 cells in the presence of serum. Compared to electroporation, the lipid-based reagent requires significantly less RNA to obtain the same level of expression from an HCV replicon. In addition, because there is low cellular toxicity associated with the lipid-based reagent, many fewer HuH7 cells are required for each transfection compared to electroporation.

Figure 1. Electroporation and Lipid-based TransIT-mRNA Transfection Kit Protocol Comparison

- Electroporation
  1. Trypsinized and harvested En5-3 cells.
  2. Washed cells 2x and resuspended in serum-free media.
  3. Added 200 µl of cells (1 x 10⁶) to 2 ml serum-containing media and added 5 µg of HCV tat2ANeo RNA replicon RNA.
  4. Electroporated cells and allowed to recover.
  5. Trypsinized and harvested En5-3 cells.
  6. Incubated 24 hours at 37°C, 5% CO₂.
  7. Harvested media containing SEAP and assayed.

- TransIT-mRNA Kit
  1. Added 1.25 µg Ntat2ANeo HCV replicon RNA to serum-free media.
  2. Added 1.25 µg RNA-Boost Reagent, 2.5 µg TransIT-mRNA Reagent, mixed and incubated 3 min.
  3. Added complete serum-containing media to 25 mm dish containing serum-containing media.
  4. Incubated 4 hours at 37°C, 5% CO₂.
  5. Harvested media containing SEAP and assayed.

Figure 2. Comparable Transfection Efficiency with the TransIT-mRNA Kit Using 75% Less RNA

En5-3 cells containing a Tat-activated reporter gene (1), were transfected with the Ntat2ANeo HCV replicon RNA as outlined in Figure 1. Expression of the Tat protein from the replicon after transfection, activates expression of the SEAP reporter in the En5-3 cells. In this experiment, SEAP activity serves as an indicator of successful transfection and the relative efficiency of the two transfection methods. As shown, the level of SEAP expression between the two methods differed by approximately 18%. Significantly, 4X less Ntat2ANeo RNA transcripts were transfected using the TransIT-mRNA Kit compared to electroporation.

Figure 3. The Boost Reagent in the TransIT-mRNA Kit is Required for Optimal Transfection Efficiency

The YFRP-IRE-Luc replicon RNA was transfected into HuH7 cells using the TransIT-mRNA Transfection Kit and the following conditions per well of a 24-well plate:
- 0.5 µg YF-Luc replicon RNA
- 0.5 µg TransIT-mRNA Reagent

Cells were harvested 24 hours post-transfection and assayed for luciferase activity.

Results and Discussion

Figure 4. Performance Comparison of Different Lipid-based RNA Transfection Reagents

Because the TransIT-mRNA Transfection Kit performed comparably to electroporation (Figure 2), we wanted to determine if other lipid-based transfection reagents were as effective at delivering viral RNA replicons. We transfected HuH7 cells in 12 well plates with 1 µg per well of the YFRP-IRE-Luc replicon RNA, and 24 hours later, we harvested the transfected cells and assayed for luciferase activity. We tested the TransIT-mRNA Kit side-by-side with DMRIE-C (Invitrogen) and TransMessenger™ (Qiagen) following the recommended protocols. We tested a range of reagent levels, and when the reagents had two components, we tested different levels of each component as suggested by the manufacturers. As shown, the TransIT-mRNA Transfection Kit outperformed both DMRIE-C and TransMessenger Reagents. In addition, the DMRIE-C and TransMessenger reagents require that the transfections be performed in the absence of serum with a media change 4-6 hours post-transfection.

Conclusions

Electroporation and the TransIT-mRNA Transfection Kit deliver viral RNA replicons with similar efficiency, but the TransIT-mRNA Kit offers several advantages over electroporation including:

1. 4X less viral RNA is required when using the TransIT-mRNA Kit compared to electroporation.
2. Because the TransIT-mRNA Kit transfects adherent cells and is not toxic, large numbers of cells are not required for transfection.
3. The TransIT-mRNA Kit protocol has fewer steps than electroporation including no trypsinization, no washing, and no transfer steps.

Compared to other commercially available RNA transfection reagents, the TransIT-mRNA kit outperforms those reagents by approximately 2-3X. It has the added advantage that transfections can be performed in the presence of serum with no media change necessary.