A Highly Efficient Method of Transfecting RAW 264.7 Cells

SUMMARY

Genlantis has developed an improved method of cationic lipid-mediated transfection, called Advanced Conjugation Enhancement (ACE), that results in a significant increase in transfection efficiency in very hard to transfect cell lines. Utilizing ACE technology with our newest transfection reagent, GenePORTER® 3000 (GP3K), we demonstrate a significant increase in transfection of RAW 264.7. Specifically, when utilizing GP3K as the transfection delivery molecule, expression levels of a *b*- galactosidase encoding plasmid are 20-fold higher than those achieved using Lipofectamine® 2000 (LF2K) (Invitrogen Corporation). Additionally, with Green Fluorescent Protein (GFP) as a substrate, the GP3K-transfected cells were substantially brighter than those transfected with LF2K. Finally, the cytotoxic effect seen with LF2K was not observed in GP3K-transfected cells. This higher transfection efficiency and lower cytotoxicity achieved with GP3K makes the transfected RAW 264.7 cells more amenable to downstream assays.

INTRODUCTION

Macrophages have been implicated in many disease states including the growth and spread of malignant tumors, inflammation, HIV infection, lysosomal storage disease, atherosclerosis, diabetes, and lupus, among others (1-5). Studies using macrophage cell lines are central to dissect the events that regulate the function of these diseased tissues. One of the most important approaches that scientists use to study these processes is gene transfer to macrophage cell lines.

RAW 264.7, a mouse macrophage cell line (6), is among the most commonly used cell types for these studies. However, gene transfer into macrophage cell lines is frequently impeded by extremely low transfection efficiencies. For this reason, gene delivery into RAW 264.7 is usually accomplished by either viral infection or electroporation. These methods result in more efficient gene delivery, but carry collateral issues that make them less attractive than lipid-mediated transfection. For example, viral infection is more problematic since the DNA cargo must conform to the size constraints of the viral vector. In addition, many viral systems require greater safety precautions and carry health risks that transfection does not. Also, producing high titer viral supernatants for each construct can also be both challenging and cumbersome.

Electroporation is a second alternative used for gene transfer into RAW 264.7. However, the mortality to the cells due to electroporation is very high, and those cells that do survive may have been physiologically altered due to the process itself. Electroporation also requires expensive instrumentation, making these experiments very costly; scaling up for high throughput efforts is also an expensive endeavor.

Genlantis' newest transfection reagent, GenePORTER 3000 improves lipid-mediated transfection into RAW 264.7 dramatically, eliminating the necessity to utilize these alternative methods for gene delivery.

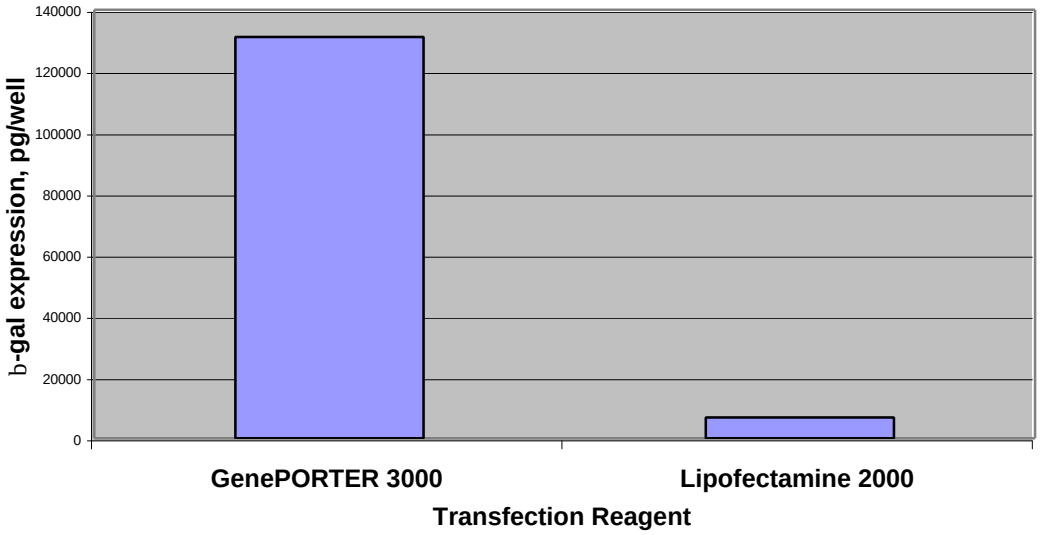
METHODS AND RESULTS

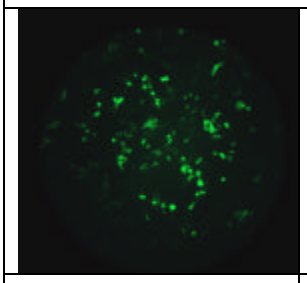
In one experiment, healthy, low passage number, log phase RAW 264.7 cells (Mouse macrophage adherent cell line; Abelson murine leukemia virus transformed) were detached using Detachin™ Solution (Genlantis Cat. No. T100100) and plated at high density (300,000 cells /500µl/well in complete medium) in a 24-well plate. The cells were incubated at 37ºC and 5% CO_2 for 30 minutes, a sufficient time to allow the cells to settle and adhere to the plate surface. Meanwhile, duplicate transfection reactions were prepared using 1µg/well gWiz ß-galactosidase plasmid DNA (Genlantis Cat. No. P010200), and either GP3K or LF2K, according to the manufacturers' recommended protocols. Just prior to adding the transfection reactions, the complete medium was removed from the cells and replaced with an equal volume of serum-free medium, Opti-MEM® I (Invitrogen Cat. No.11058-021). The transfection reactions (lipoplexes) were added directly to the cells and incubated at 37ºC in 5% CO_2. After 4 hours, an equal transfection volume of complete medium containing 20% serum was added.

The transfected cells were lysed and assayed for β-galactosidase activity approximately 48 hours post-transfection (ONPG Assay Kit. Genlantis Cat. No. A10200K). The results show that the β -galactosidase activity for RAW 264.7 cells transfected with GENEPORTER 3000 was approximately 20X higher than those transfected with LF2K (**Figure 1**).

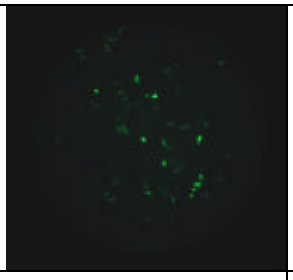
In a similar experiment, freshly detached RAW 264.7 cells were plated directly on glass coverslips in 12-well tissue culture plates at a density of 300,000 cells/well. The reporter plasmid, gWiz GFP (Genlantis Cat. No. P040400) was transfected with either GP3K or LF2K according to the manufacturer's recommended protocols. The cells were screened 48 hours post-transfection for GFP expression with a fluorescent microscope using a FITC laser (Ex 480/30, DM 505, BA 535/40). The results indicate higher transfection efficiency, robust GFP expression and healthy morphology for cells transfected with GP3K (**Figure 2**) compared to those transfected with L2K. This notable increase in GFP expression may reflect either an increased number of plasmids gaining entry into the nucleus and/or better expression mediated by lower cytotoxicity (**Figure 3)**.

Figure 1: Raw 264.7 ONPG Assay Results





A) GenePORTER 3000



B) Lipofectamine 2000

Figure 2: Whole field 20X magnification view of GFP expression in RAW 264.7 cells transfected with gWIZ GFP and **A)** GenePORTER 3000 or **B)** Lipofectamine 2000.

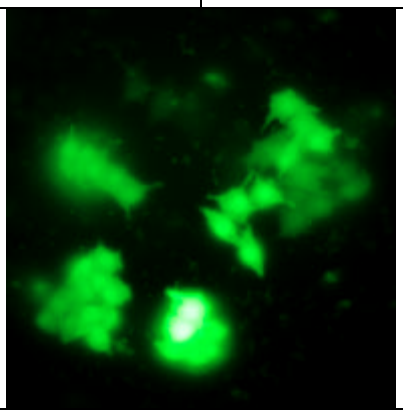


Figure 3: 60X magnification view of GFP expression in RAW 264.7 cells transfected with gWIZ GFP and GenePORTER 3000

DISCUSSION

RAW 264.7 is a mouse macrophage cell line that is very difficult to transfect using any commercially available cationic lipid or polymer delivery reagent. Because of this low efficiency, researchers often turn to more expensive and cumbersome methods to transfect RAW 264.7, such as electroporation or viral

delivery. Utilizing a new transfection reagent, GenePORTER 3000, that exploits a novel delivery technology – Advanced Conjugation Enhancement (ACE) - we have demonstrated that lipid-mediated transfection into RAW 264.7 increases by a factor of 20 when compared with the most commonly used cationic lipid, Lipofectamine 2000; the improved efficiency combined with very low cytotoxicity provides a very useful alternative for gene delivery into RAW 264.7

References

1. Biswas, S.K., Sica, A., and Lewis, C.E. (2008). J. Immunol. 180:2011-2017.

2. Victoria, E.O., Xu, X, Koska, J., Francisco, A.M., Scalise, M., Ferrante Jr., A.W., and Krakoff, J. (2009) Diabetes 58: 385-393.

3. Lai, J., Bernhard, O.K., Turville, S.G., Harman, A.N., Wilkinson, J., and Cunningham, A.L. (2009). J. Biol. Chem. in press.

4. Liu, J., Thewke, D.P., Su, Y.R., Linton, M.F., Fazio, S., and Sinensky, M.S. (2005). Atherosclerosis, Thrombosis, and Vascular Biology 25:174-179.

5. Rogers, N.J., Lees, M.J., Gabriel, L., Maniati, E., Rose, S.J., Potter, P.K., and Morley, B.J. (2009). J. Immunology 182:1982-1990.

6. McKernan, L.N., and Largen, M.T. (1983) Cell Immunology 80:84-96.

Genlantis
A division of Gene Therapy Systems, Inc.
10190 Telesis Court
San Diego, CA 92121

Phone:
888-428-0558
858-457-1919

Fax:
858-623-9494

Website:
www.genlantis.com