# Determination of the Firefly Luciferase mRNA Concentration and Encapsulation Efficiency in Protein Nanoparticles Using RiboGreen Assay

under the direction of

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Abstract

Messenger RNA (mRNA) has been used as a powerful therapeutic in protein gene therapy carriers and is used for infectious diseases. Novel delivery systems, such as lipid protein nanoparticles and reagents, are aimed at protecting mRNA from degradation. To quantify mRNA and to find its Encapsulation Efficiency, RiboGreen, a proprietary fluorescent RNA binding dye, was used for the detection and quantitation of RNA. The purpose of this study was to optimize a Ribogreen Assay for determination of Fluc mRNA Encapsulation Efficiency in protein nanoparticles, including Lipofectamine Messenger Max (LFMM) and solid Lipid Nanoparticles (LNPs). In the study, the effects of assay conditions associated with detergent (Triton) extraction of the mRNA were analyzed.

We found that increasing the volume of LFMM to the weight of the mRNA ratio from 1.5 to 6 ul/ug resulted in the complete encapsulation of mRNA. Our initial 25 percent underestimate of free mRNA without LFMM (3.5 vs 4.7) was found to be due to poor replicates in the standard curve, and differing conditions in the sample vs standard (OptiMEM, Triton). By using identical conditions in the sample and standard and ensuring duplicate repeatability (5 percent), the 25 percent underestimate was reduced to 6 percent. The optimal carrier condition range in terms of carrier state, volume to weight ratio of carrier to contents, and time duration was determined. Future applications include finding the exact optimal setting (vector type, reagent components, and ratio) for the absolute highest encapsulation efficiency of mRNA possibly in any protein carrier.