Introduction

Bacterial luciferase is the light producing catalyst in a bacterial cell. This reaction is directly dependent on the presence of adenylate triphosphates (ATP). We have recently developed a variant of luciferase with an internal 57 amino acid deletion from Culex cruciata (Japanese mosquito). This new gene codes for a modified amino acid sequence which exhibits improved expression levels in mammalian cells, red-shifted emission wavelengths (560 to 615 nm) as well as increased thermal stability, while still utilizing Coelenterazine as an ATP substrate.

This new recombinant enzyme was purified to 98% homogeneity from both transformed E. coli bacteria and HEK 293 mammalian cells using "affinity chromatography as the initial purification step, followed by "affinity chromatography against an immobilized His stalk in A TP dependent assays. The purified recombinant luciferase was used to develop a number of new bioluminescent assays useful in measuring the activity of a variety of bioluminescent systems as well as the cytotoxic effects of several chemotherapy drugs (Disparate and Cytokine). When added to either HEK or human RET cells in solution, the assay utilized the purified luciferase enzyme to sensitize detectable bacterial cell number down to 1 x 10^3 cells and adapted the method for use in high-throughput antibiotic screening protocols. By using this luciferase as a bioluminescent reporter in mammalian cell systems, the results showed luciferase activity (light) in human HEK 293 cells could be quantitated by directly monitoring the increase in luminescence from activation of luciferase expression. Finally, since this new luciferase has a red-shift in light emission, it was utilized in multiplexed assay conditions with other green (fleatox) or blue-emitting (luciferin) bioluminescents in a number of coupled bioluminiscence assays. Moreover, application of this new luciferase for use in vivo imaging was explored.

Methods

The assay was performed in a 96-well format with Coelenterazine substrate in a 5:1 ratio with ATP. Lysis was performed with 100 µl extraction buffer and proteinase K. Lysis was continued overnight at 40ºC. All cell extracts were frozen and general proteinase K treated overnight in 1:1000 solution.

Luciferase assay

In general, the luciferase assay was performed in a 96-well plate format. Following cell lysis with icecold 10 mM Tris-HCl (pH 7.5) and the addition of 100 µl of lysis buffer to cell culture plate wells, the plate was incubated at room temperature for 5 minutes before the addition of 48 µl of luciferase reaction buffer to the 96-well plate. The luciferase reaction was then performed by placing the plate in an Ikonick Luminescent Instrument 96-well plate reader. Readings were performed in triplicates. The assay was performed on an Ikonick Luminescent Instrument 96-well plate reader. Readings were performed in triplicates. The assay was performed on a 96-well plate reader.

Results

Figure 1: Luciferase activity in mammalian cell lines. Luciferase activity was measured using luciferase assay. 0.25 ml of cell extract (400,000 cells/ml) with 48 µl of ATP and 1% Triton x100 was used. The luciferase activity was measured using a spectrophotometer and the absorbance was read at 7873. ATP was added to the mixture and then the luciferase activity was measured using a spectrophotometer.

Figure 2: Comparison of recombinant Ruiuliferase luciferase assay in mammalian and bacterial cells. 2 x 10^6 NIH-3T3 cells were transfected in 0.2 ml of luciferase assay. The luciferase assay was performed on a 96-well plate reader. Readings were performed in triplicates. The assay was performed on a 96-well plate reader.

Figure 3: Measurement of bacterial cell number by luminescence with recombinant luciferase. 2 x 10^6 NIH-3T3 cells were transfected in 0.2 ml of luciferase assay. The luciferase assay was performed on a 96-well plate reader. Readings were performed in triplicates. The assay was performed on a 96-well plate reader.

Figure 4: Measurement of bacterial cell number by luminescence with recombinant luciferase. 2 x 10^6 NIH-3T3 cells were transfected in 0.2 ml of luciferase assay. The luciferase assay was performed on a 96-well plate reader. Readings were performed in triplicates. The assay was performed on a 96-well plate reader.

Figure 5: Measurement of bacterial cell number by luminescence with recombinant luciferase. 2 x 10^6 NIH-3T3 cells were transfected in 0.2 ml of luciferase assay. The luciferase assay was performed on a 96-well plate reader. Readings were performed in triplicates. The assay was performed on a 96-well plate reader.

Figure 6: Measurement of bacterial cell number by luminescence with recombinant luciferase. 2 x 10^6 NIH-3T3 cells were transfected in 0.2 ml of luciferase assay. The luciferase assay was performed on a 96-well plate reader. Readings were performed in triplicates. The assay was performed on a 96-well plate reader.

Discussion and Conclusions

After completing the Heterologous recombinant luciferase in bacterial cell, it was purified to homogeneity and activity with the additional advantage of ready availability of a mammalian luciferase in a variety of expression systems. The results showed luciferase activity (light) in bacterial cells could be quantitated by directly monitoring the increase in luminescence from activation of luciferase expression. The assay was performed on a 96-well plate reader. Readings were performed in triplicates. The assay was performed on a 96-well plate reader.

Luciferase activity was measured using a spectrophotometer and the absorbance was read at 7873. ATP was added to the mixture and then the luciferase activity was measured using a spectrophotometer.

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