

Optimization of Cell lysis-reagent solution by Statistical Methods

Ji-Hye Kim¹, Eun-Young Kwak², Ji-Ho Park², Hye-Mee Park², Young-Bum Kim²,
Woo-Jin Chang¹, Jin-Hwan Kim², Eun-Ki Kim^{1,2}

¹Center for Advanced Bioseparation Technology, ²Dept. of Biol. Eng., Inha Univ., ³M&B GreenUs
TEL: +82-32-860-7514, FAX: +82-32-874-4046

Abstract

The detection of microbes in drinking water and foods is very important to maintenance of food safety. Therefore the microbial detection method should be developed. Bioluminescent assay method which employs the ATP dependent luciferin-luciferase reaction is a reliable technique to measure the number of bacterial cells.

The extraction of bacterial ATP should be preceded to detect microbes in drinking water and foods making use of bioluminescent assay methods in microfluidic device. The general method of extracting microbial ATP is sonication. The reagents of lysis are surfactants, TCA, lysozyme etc.

It was selected to the reagent improved ATP extracting efficiency, mixing effects and rarely effected to luciferase. Extraction of the intracellular ATP by the cells account using the optimized lysis reagent.

We showed lysis, BKC(benzalkonium chloride) and lysozyme were high efficiency materials at cell - lysis, Plackett-Burman Design used. The optimum concentration was 10ppm Lysozyme, 250ppm BKC in Tricine buffer(pH7.8), respectively. using by Response surface methodology. The bioluminescence, ATP extraction efficiency was a 3-fold higher at low account of cells than in a surfactant that is general method.

References

1. A. THORE,¹* A. LUNDIN,¹† ANSEHN S², Firefly Luciferase ATP Assay as a Screening Methods for Bacteriuria (1983), *Journal of Clinical Microbiology*, Vol(17), 218-224.
2. LAURA SELAN,¹ FRANCESCA BERLUTTI,² CLAUDIO PASSARIELLO,¹MARIA C. THALLER,²* AND GIULIO RENZINI¹, Reliability of a Bioluminescence ATP Assay for Detection of Bacteria (1992), *Journal of Clinical Microbiology*, Vol(30), 1739-1742.