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4-Trifluorobenzamidoxime as a specific fluorogenic reagent for cytosine

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[Introduction] Cytidine is converted to uridine by cytidine deaminase in mammals, and almost no cytosine is released in the catabolic pathway of pyrimidine nucleotides. However, it has been mentioned that high level of cytosine is found in urine of children having immunodeficiency. Conventional methods for the detection of cytosine in biological samples have utilized high performance liquid chromatography, tandem MS system and carbon nanotubes film. Although these methods are superior for the determination of cytosine in a complex sample, these methods require special instrumentations and high costs for the analysis. Therefore, a facile and high throughput technique to quantify cytosine has been demanded. In this study, we have developed a fluorescence (FL) derivatization reaction of cytosine using 4-trifluorobenzamidoxime (4-TFMBAO) as a fluorogenic reagent. The reaction requires heating for several minutes, and cytosine concentration can be quantified by measuring the resulting FL with a spectrofluorometer.

[Methods] To a solution of cytosine were added 4-TFMBAO, $K_3[Fe(CN)_6]$ and dimethylformamide (DMF) and KOH, and the mixture was heated at 100°C for 10 min. The FL intensity of the mixture was measured with a spectrofluorometer.

[Results and Discussion] We found that the addition of DMF into the reaction mixture in combination with 4-TFMBAO dramatically enhance the FL intensity from cytosine. Under the optimized reaction conditions, strong FL was produced with cytosine, and negligible level of FL intensities were observed with uracil, adenine, guanine and thymine. Furthermore, other biological samples such as nucleobase analogues, nucleos(t)ides, amino acids and sugars exhibited no FL. The purposed method is easy and simple, and could be applicable to the detection of cytosine in biological samples.