Supplementary Information

Separation of adenine, adenosine-5'-monophosphate and adenosine-5'-triphosphate by fluidic chip with nanometre-order diameter columns inside porous anodic alumina using an aqueous mobile phase

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Fluidic chip

Three substrates (4×6 cm) were made by cutting Glycol-modified polyethylene terephthalate (PETG, Mitsubishi Rayon Co., Ltd.) plate, and channels were carved in the substrates. Five PAA membranes (Anodisk, 100 nm diameter pores, 60 μm thick, Whatman) were put between middle and upper substrates. These substrates and PAA membranes were heated at 140 °C in an oven for a few minutes. After heating, in order to combine substrates, force was applied on the upper substrate.

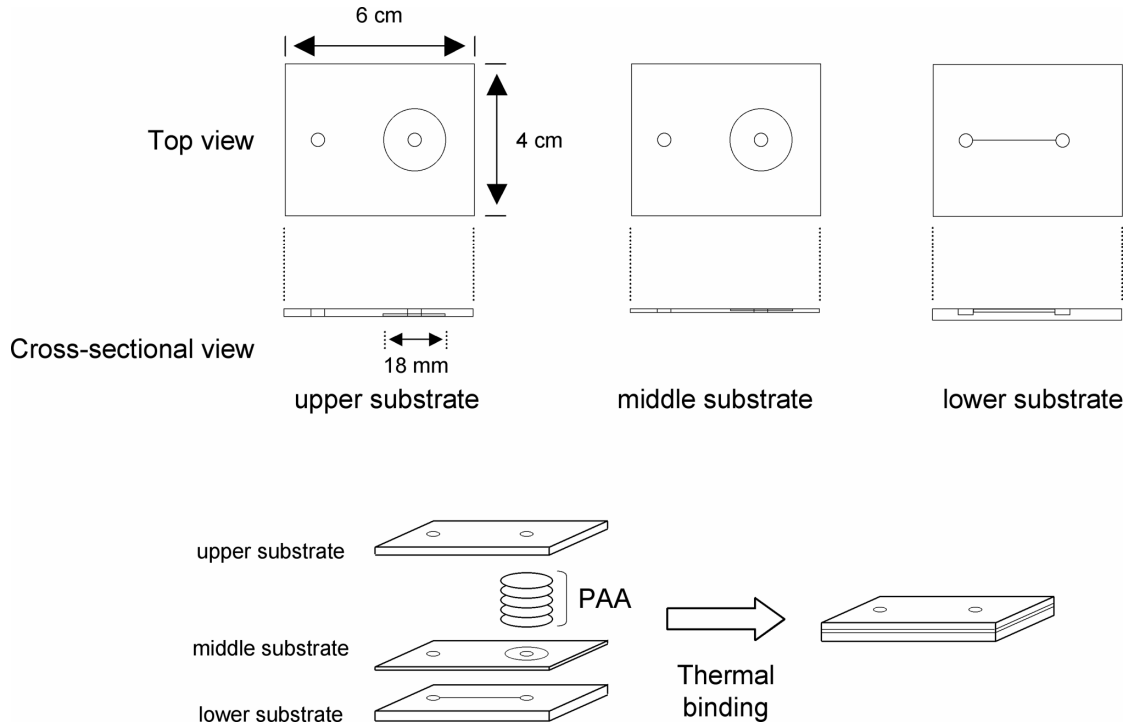


Figure S1 Schematic representation of making fluidic chip.

Chemicals

Adenine sulfate (Alfa Aesar), Adenosine 5'-monophosphate, disodium salt (Oriental Yeast Co.,Ltd.), Adenosine 5'-diphosphate, monosodium salt (Wako Pure Chemical Industries Ltd.), Adenosine 5'-triphosphate, disodium salt trihydrate (MP Biomedicals), Sodium Carbonate (Wako Pure Chemical Industries Ltd.) were used without further purification.

Chromatographic measurement

In chromatographic measurements, Model G1311A HPLC pump, Model 1313A autosampler, and Model G1315B UV detector (Agilent Technologies) were used. Water was obtained from a TORAYPURE LV-05 system (Toray Industries). A flow-rate of 0.4 mL/min was used with the following linear gradient: 0 min, 100% purified water; 10 min, 100% 8×10^{-4} M Na_2CO_3 aqueous solution (pH 9.9). The injection volume was 20 μL. The mobile phase was degassed by helium during measurements.

Residual amount measurement

Water was obtained from a TORAYPURE LV-05 system (Toray Industries). The PAA membrane was immersed in a glass bottle filled with 10 ml of 2.5×10^{-5} M solute in a buffer solutions. As buffer solutions, 8×10^{-4} M $NaHCO_3$ (pH 7.3) and 8×10^{-4} M Na_2CO_3 (pH 9.8) were used. The glass bottle was immediately closed with a cap to minimize vaporization and shaken gently on a reciprocating shaker (TAIYO RECIPRO SHAKER SR-II) at 25°C. After shaking the sample for 12 hr, UV absorbance of the solution was measured, and concentration of solute (C_e) was estimated. C_0 was also estimated by repeating the above procedure without a PAA membrane. The detection wavelength was 265 nm for adenine and 260 nm for AMP, ADP and ATP.

Estimation of resolutions

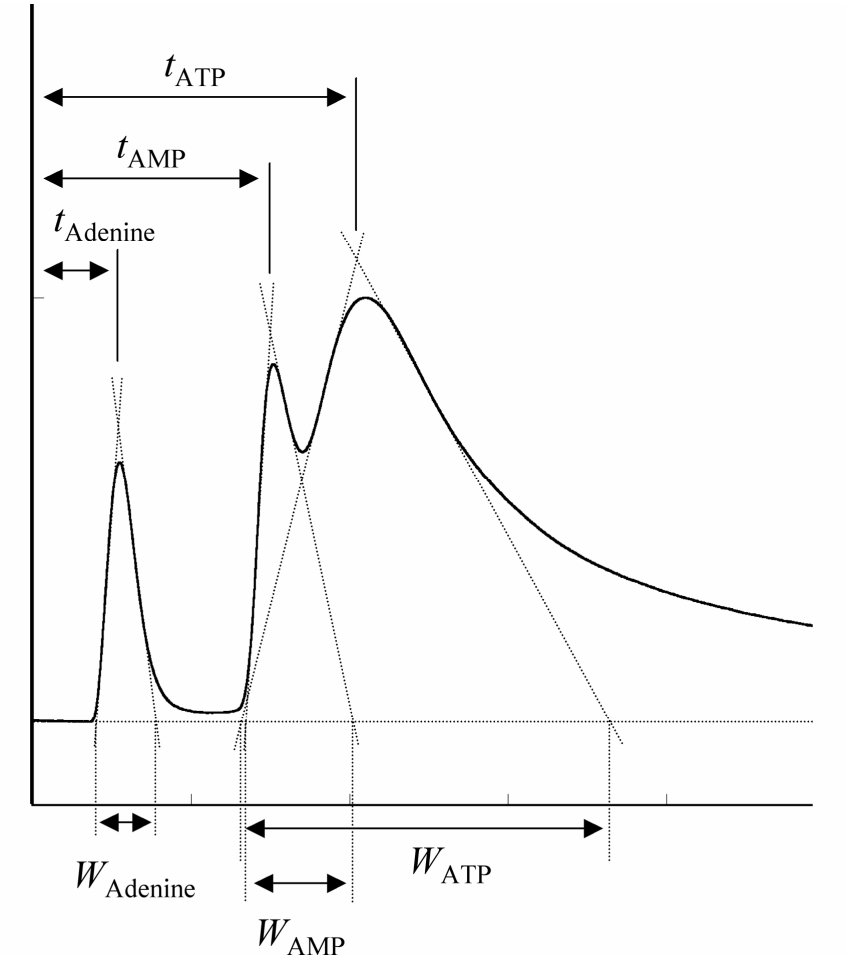


Figure S2 Estimation of peak width

Resolutions (R_s) between two peaks were given by following equation:

$$R_s(\text{A-B}) = \frac{2(t_B - t_A)}{W_A + W_B}$$

where t is retention time, and W is peak width. Table S1 summarizes the values of resolution.

Table S1 Values of resolution

	Adenine - AMP	Adenine - ATP	AMP - ATP
R_s	1.83	1.11	0.37

Successive runs

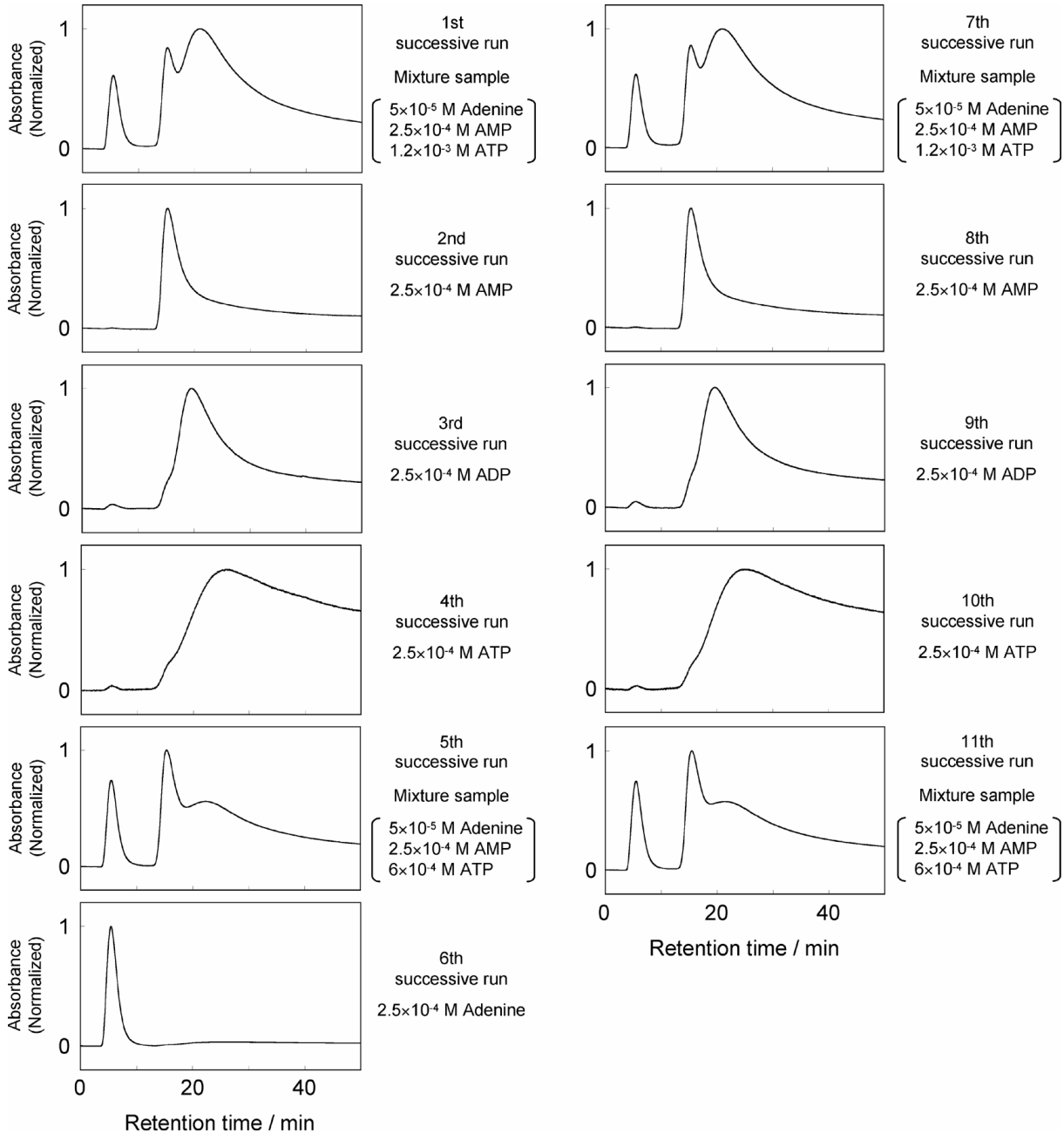


Figure S3 Chromatograms of successive runs. To determine the reproducibility of chromatograms for each solute, chromatograms of each solute were measured one after the other. A flow-rate of 0.4 mL/min was used with the following linear gradient: 0 min, 100% purified water; 10 min, 100% 8×10^{-4} M Na_2CO_3 aqueous solution (pH 9.9). The injection volume was 20 μL. The mobile phase was degassed by helium during measurements. The detection wavelength was 280 nm.

Chromatograms without fluidic chip

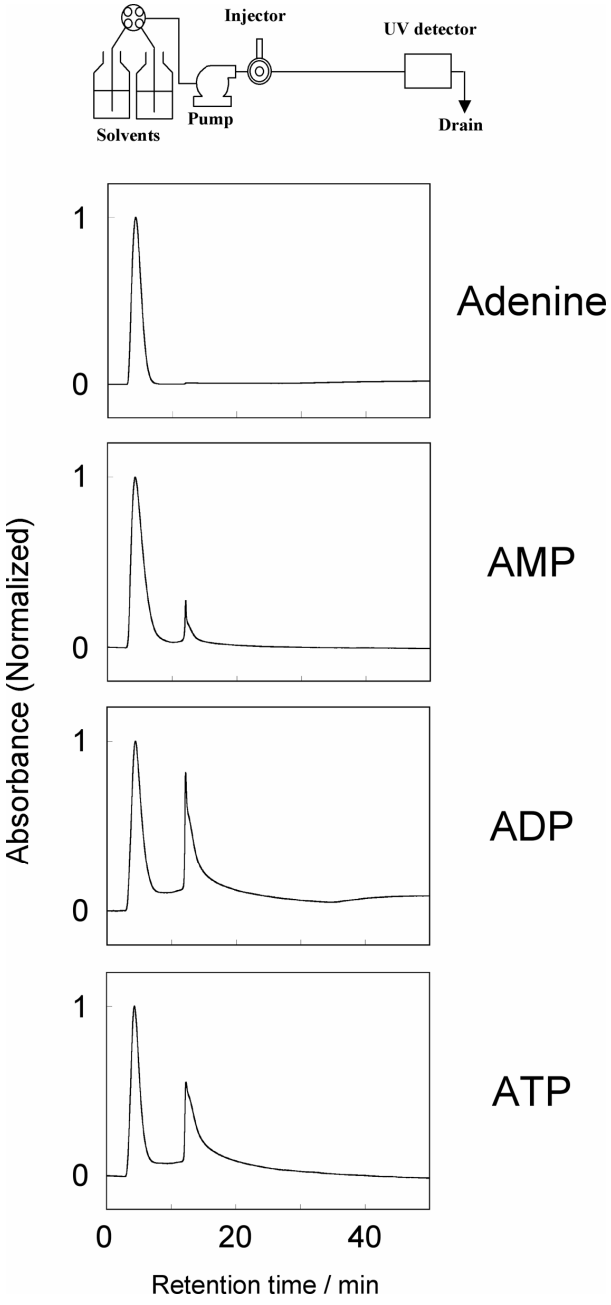


Figure S4 Chromatograms of adenine, AMP, ADP and ATP without fluidic chip. A flow-rate of 0.4 mL/min was used with the following linear gradient: 0 min, 100% purified water; 10 min, 100% 8×10^{-4} M Na_2CO_3 aqueous solution (pH 9.9). The injection volume was 20 μL. The mobile phase was degassed by helium during measurements. The detection wavelength was 280 nm.

In the case of AMP, ADP and ATP, a second peak appeared around 12.1 min. This peak was due to partial adsorption of the solutes in the chromatographic lines (stainless and Teflon tubes).

Chromatograms of adenine and AMP are compared in figure S5. Because adenine hardly interacts with either the PAA membrane or the chromatographic lines, the difference of retention time between “Adenine (without chip)” and “Adenine (with chip)” corresponds to the void volume of the chip. This time difference (5.5 – 4.3 = 1.2 min) is smaller than the difference of retention time

between “AMP second peak (without chip)” and “AMP (with chip)” (15.3 – 12.1 = 3.2 min). This result indicates that all AMP molecules adsorbed once in PAA membranes.

Portions of the AMP, ADP and ATP adsorbed on chromatographic line at the beginning of a run. As the pH increased during a run, the solutes desorbed from the chromatographic lines. The desorbed solutes then adsorbed to the PAA membranes. Eventually, the retention time is determined by elution from the PAA membranes. Therefore, in this fluidic chip system, it is unnecessary to consider the effect of partial adsorption to the chromatographic lines.

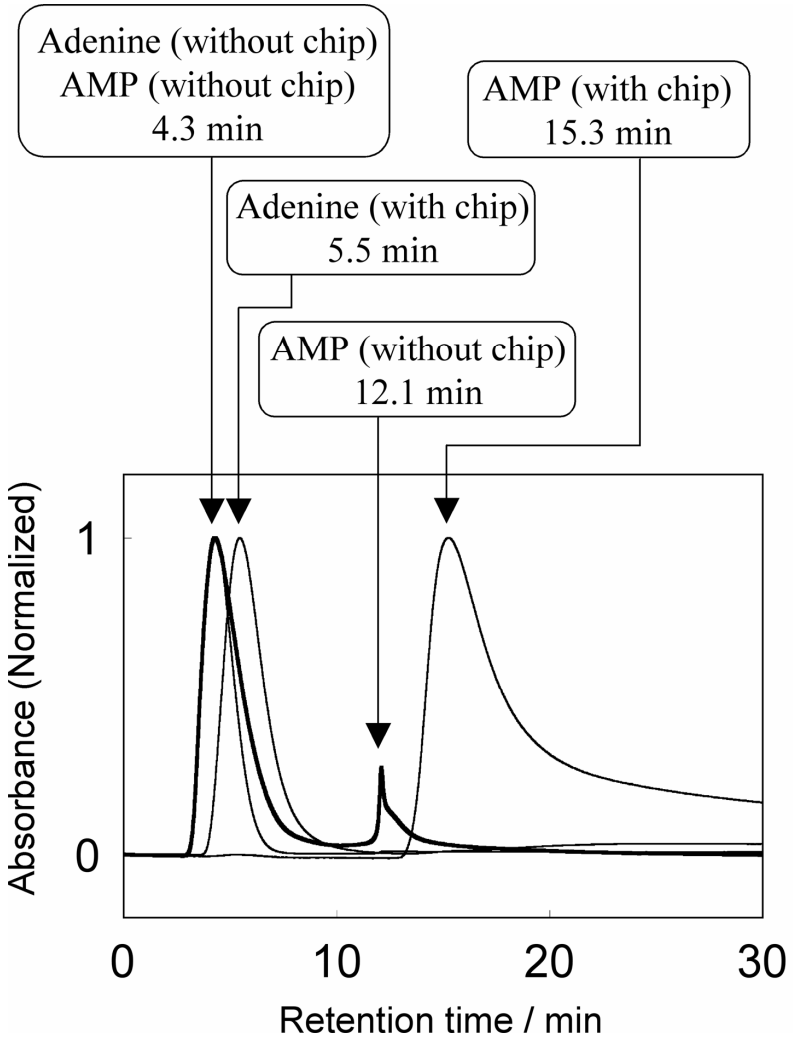
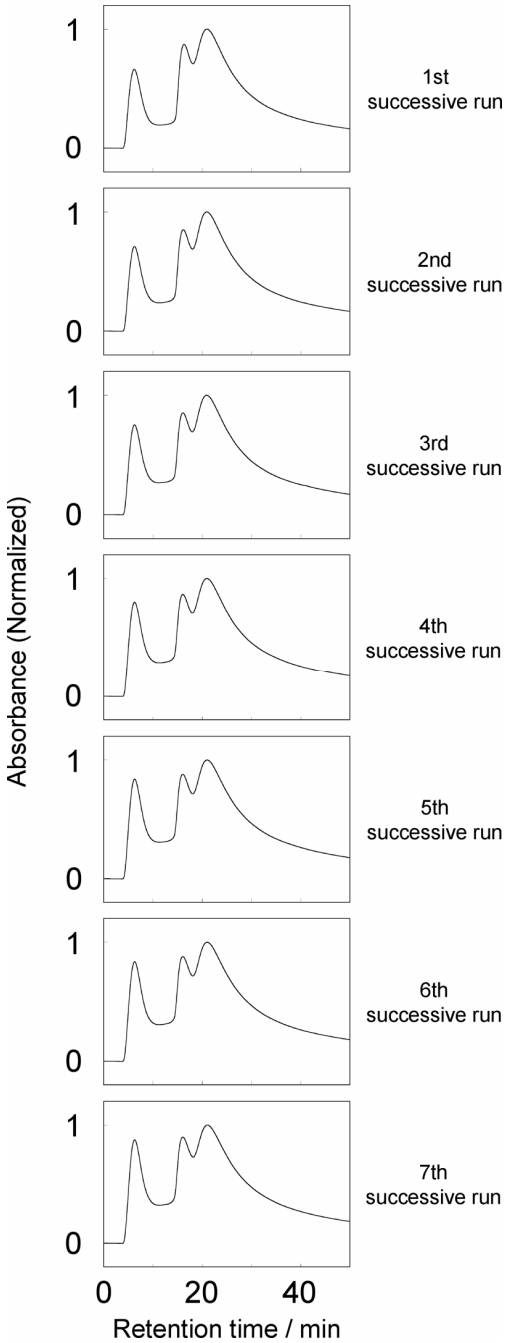


Figure S5 Comparison of chromatograms of adenine and AMP with or without a fluidic chip.

Estimation of CV (Coefficient of Variation) of retention time



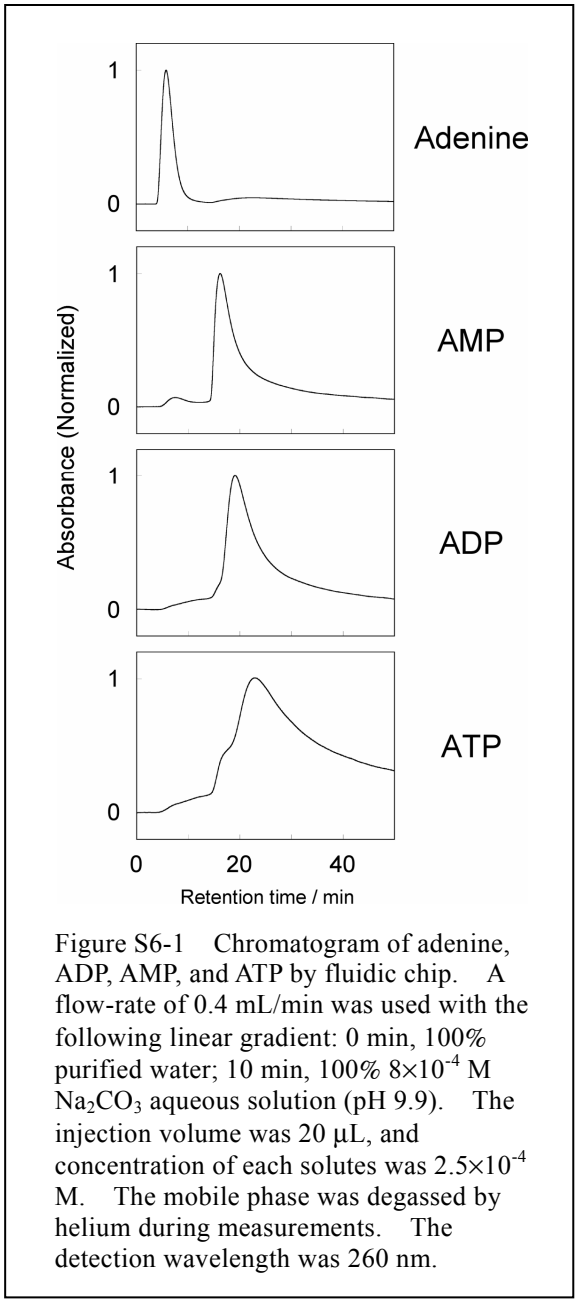


Figure S6-1 Chromatogram of adenine, ADP, AMP, and ATP by fluidic chip. A flow-rate of 0.4 mL/min was used with the following linear gradient: 0 min, 100% purified water; 10 min, 100% 8×10^{-4} M Na_2CO_3 aqueous solution (pH 9.9). The injection volume was 20 μL, and concentration of each solutes was 2.5×10^{-4} M. The mobile phase was degassed by helium during measurements. The detection wavelength was 260 nm.

Figure S6-2 Chromatograms of successive runs. Chromatograms of solutes were measured successively. A flow-rate of 0.4 mL/min was used with the following linear gradient: 0 min, 100% purified water; 10 min, 100% 8×10^{-4} M Na_2CO_3 aqueous solution (pH 9.9). The injection volume was 20 μL, and concentrations of adenine, AMP and ATP were 5×10^{-5} M, 2.5×10^{-4} M and 7.0×10^{-4} M, respectively. The mobile phase was degassed by helium during measurements. The detection wavelength was 260 nm

(In this experiment, we made the fluidic chip again and measured the chromatogram of mixture sample successively. Since fluidic chips were made by hand, retention time and resolution were slightly different from results of previous chip.)

CV was given by following equation:

$$CV = \frac{S}{A} \times 100$$

where S is standard deviation, and A is average. Table S2 summarizes the values of CV.

Table S2 Values of CV of retention time

	Adenine	AMP	ATP
CV	0.68	0.58	0.18