Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting information

High-throughput single-molecule bioassay using micro-reactor arrays with a concentration gradient of target molecules

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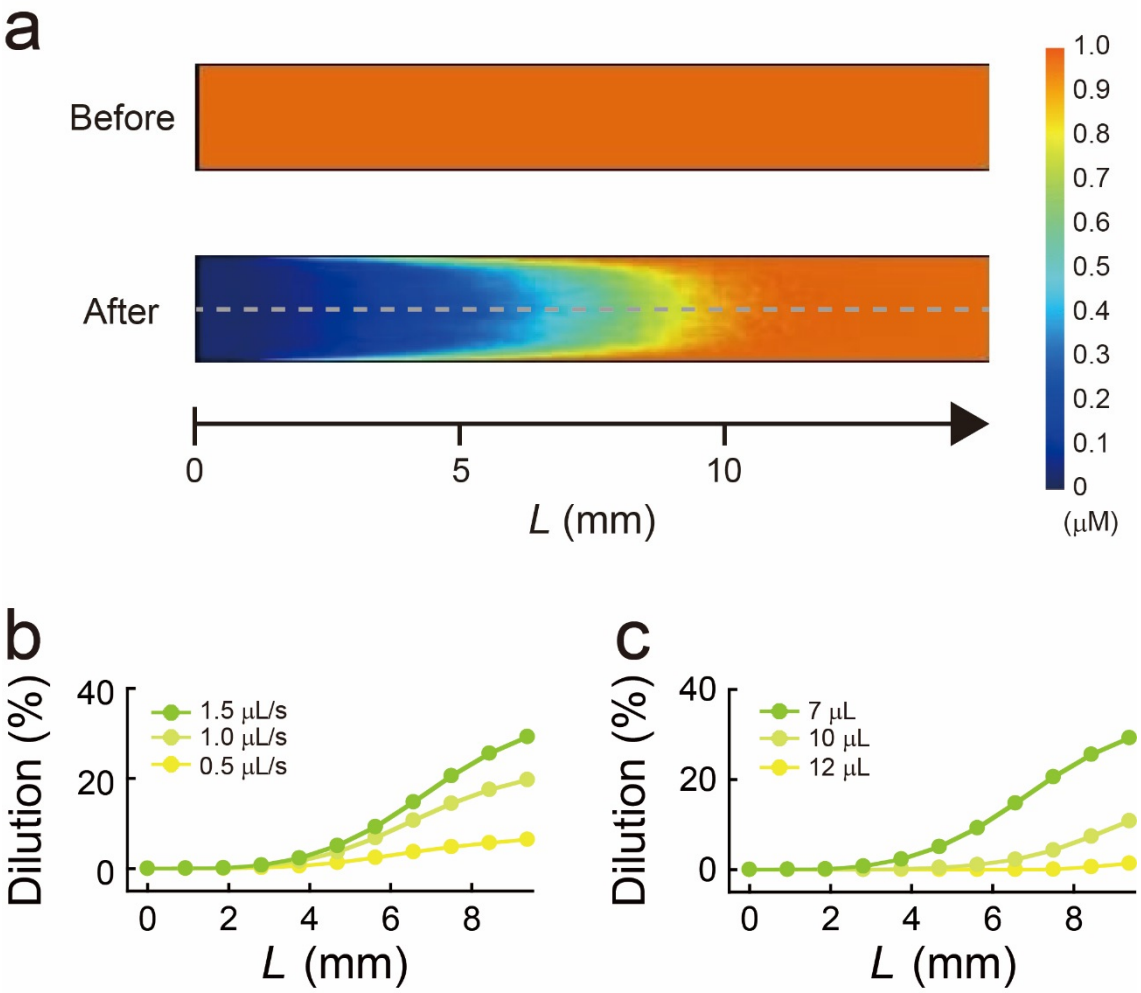

Fig. S1. Numerical simulation of advection diffusion based on finite element method (**a**) Color map of concentration gradient of target molecules on the bottom surface of flow channel before (top) and after (bottom) injection of the second aqueous solution. The left and right end were the inlet and outlet of flow channel, respectively. The flow rate and volume of the second buffer solution were 1.5 μL/s and 7 μL, respectively. The diffusion coefficient of the target molecule was 400 $\mu m^2/s$. The concentration of target molecules before injection of the second buffer solution was 1 μM. The numerical simulation was conducted using COMSOL Multiphysics. (**b and c**) Simulated dilution rates at each *L* along the grey dashed line in Fig. S1a are plotted against the flow rate (**b**) or the volume of the dilution buffer (**c**).

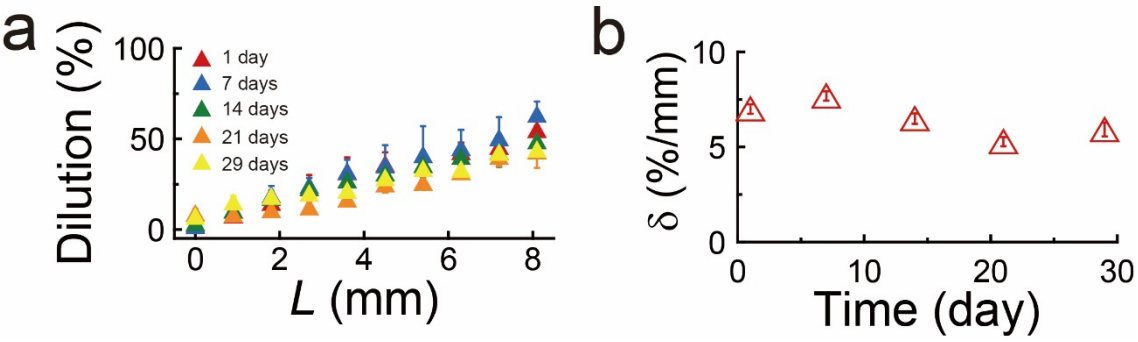

Fig. S2. Dilution rate against the aging period

(**a**) Dilution rate of Alexa 488 at each *L* are plotted against the aging period. The low rate and the volume of the second buffer solution used to generate the concentration gradient were 1.5 μL/s and 7 μL, respectively. (**b**) Slopes of dilution rate in Fig. S2a are plotted against the aging-period.

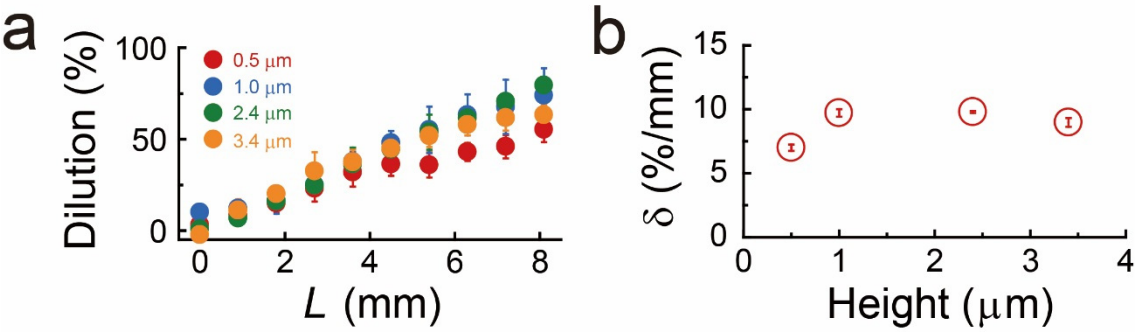

Fig. S3. Dilution rate against the height of micro-reactors

(a) Dilution rate of Alexa 488 at each *L* are plotted against the height of micro-reactors. The low rate and the volume of the second buffer solution used to generate the concentration gradient were 1.5 μL/s and 7 μL, respectively. (b) Slopes of dilution rate in Fig. S3a are plotted against the height of micro-reactors.

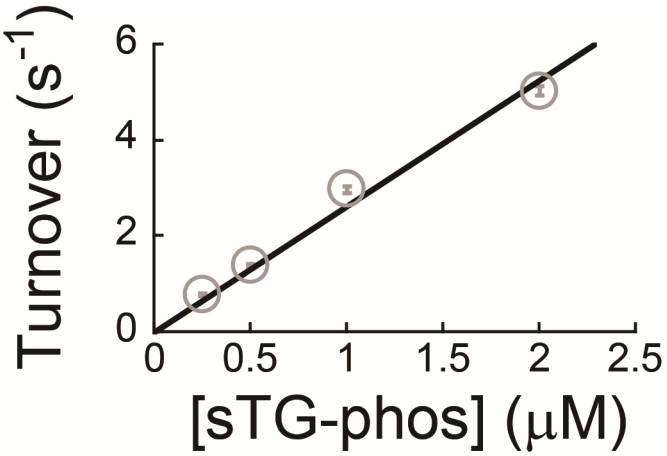

Fig. S4. Bulk assay of ALP, as determined by spectrometry

Turnover of ALP was plotted against [sTG-phos]. The black line represents the linear regression. Results were obtained from 3 independent experiments, and average values (circles) are plotted with SDs (bars).

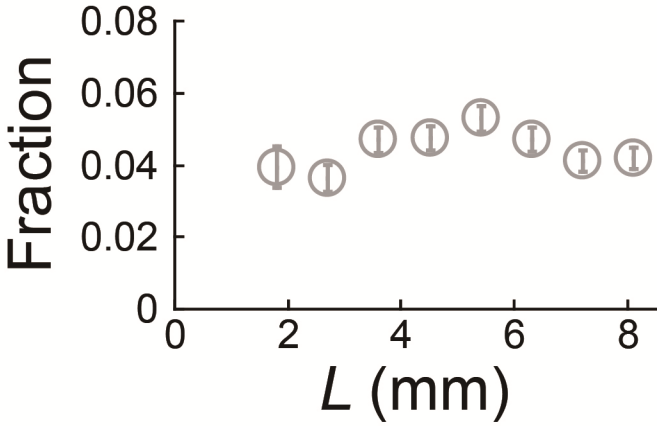

Fig. S5. Fraction of green fluorescent reactors

The fraction of reactors with green fluorescence, sTG, was plotted against the distance from the access port, *L*. Results were obtained from 3 independent experiments, and average values (circles) are plotted with SDs (bars).

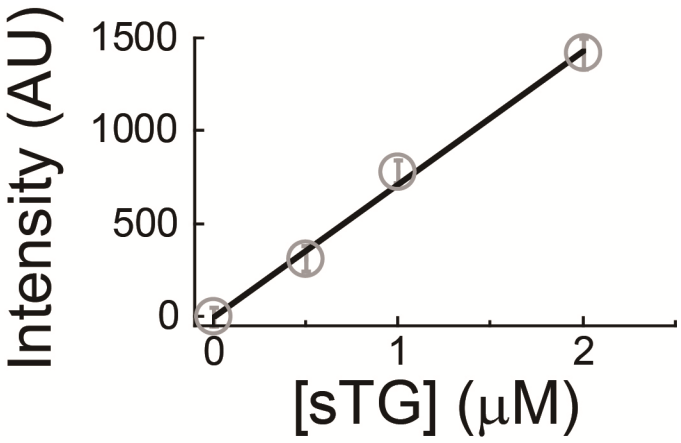

Fig. S6. Fluorescence intensity of sTG under a microscope

Dependence of the fluorescent intensity of sTG on its concentration. The black line represents the linear regression. Results were obtained from 3 independent experiments, and average values (circles) are plotted with SDs (bars).