Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information for

Rapid colorimetric analysis of multiple microRNAs using encoded hydrogel microparticles

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I. Estimation of the SA-PE concentration bound in PSF particles

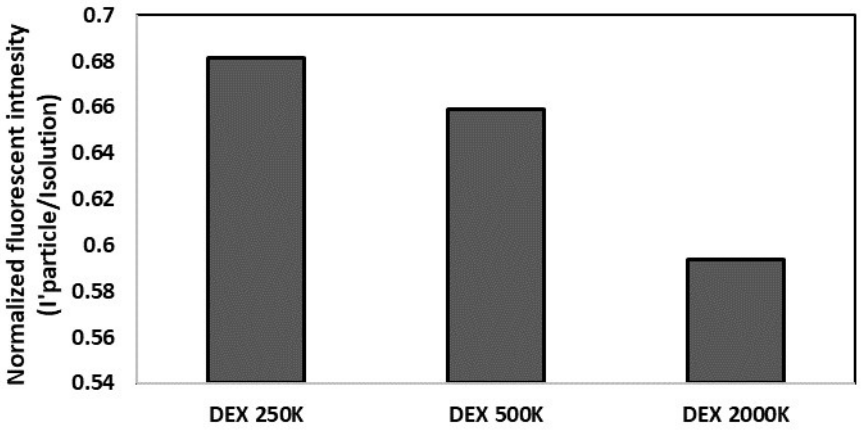

Figure S1. Fluorescent signals of PEG hydrogel microparticles after diffusion of dextran-FITC. Each data bar represents an average signal intensity.

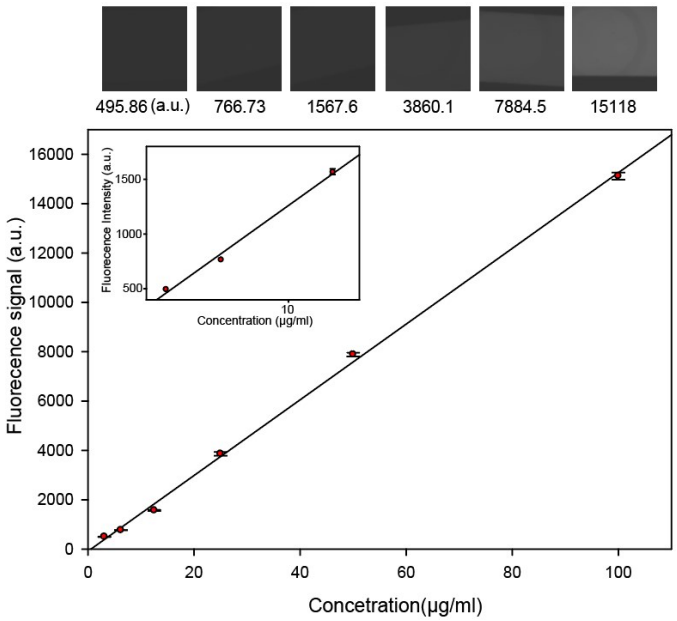

Figure S2. A calibration curve of streptavidin-phycoerythin (SA-PE) solution in 24 μm-height channel. Each data point represents average signal of SA-PE solution.

To validate the conjugation of SA-PE instead of SA-AP for the calculation of AP concentration in microparticles, we performed a diffusivity test using hydrogel particles and fluorescent-labeled biomolecules (Dextran-FITC) of various molecular weights (Figure S1). As a result, there was no big difference in the diffusion rate of biomolecules below 500 kD. Considering that SA-AP and SA-PE have molecular weights of 200 kD and 360 kD each, we can estimate that diffusion rate would not affect the binding rate of the materials within hydrogel microparticles.

A calibration curve of SA-PE was generated (Figure S2) to calculate the amount of streptavidin-phycoerythrin (SA-PE) bound to microparticles. By interpolating the fluorescent intensity of SA-PE labeled PSF microparticles (1806 a.u.) to the linear region of the calibration curve, we obtained 12.3 μg/ml as a corresponding value of SA-PE concentration. SA-PE has a molecular weight of 360 kD, and we can convert the value into molar concentration. As a result, the SA-PE concentration (≈ SA-AP concentration) in the particles was calculated to be about 33 nM.

II. Particle-to-particle variations of colorimetric signal

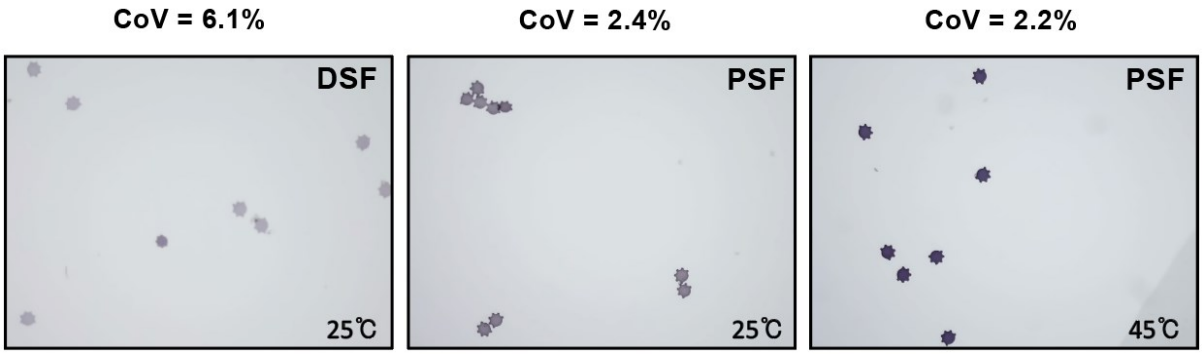

Figure S3. Images of color labeled DSF and PSF hydrogel microparticles after enzyme reaction in different enzyme reaction temperatures. Coefficient of variation (CoV) values of the colorimetric signal intensity in each case are represented above the images.

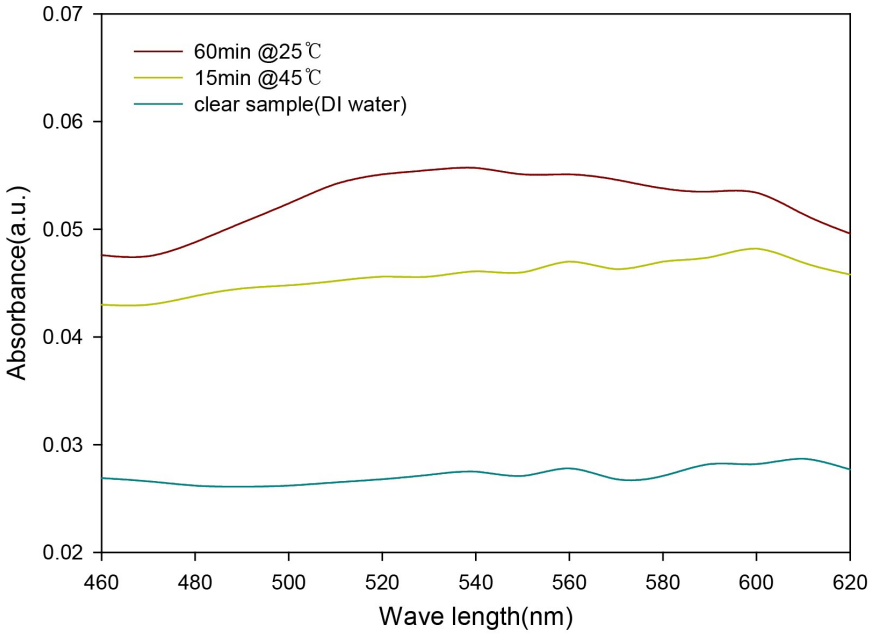

Figure S4. Absorbance spectra for background solution after BCIP/NBT reaction.

III. Comparison of chromogenic background signal

During BCIP/NBT reaction, the background signal can be developed by means of an amplifiable reaction of enzyme either remaining in solution or bound on the surface of the microparticles which cannot be completely removed during particles rinsing. We measured the background signals of two BCIP/NBT reaction condition: 1) 60 min at 25 °C, 2) 15 min at 45 °C (Figure S3). After the BCIP/NBT reaction, absorbance of solution was measured

at two cases. The wavelength of maximum absorbance for NBT-formazan is about 560nm. At reaction with BCIP/NBT by 60 min at 25 °C, background solution showed about 1.3-fold higher signal compared to BCIP/NBT reaction by 15 min at 45 °C, representing 30% increased background noise.

IV. Potential to further reduction of reaction time

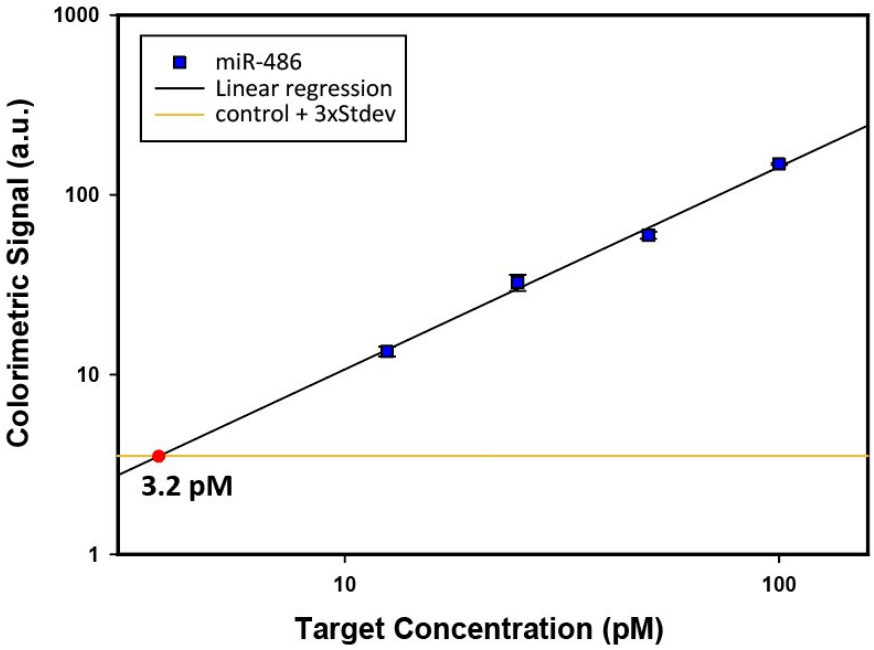

Figure S5. A standard calibration curve of miR-486 with a reduced total assay time.

Table S1. Base sequence of miRNA targets, probes, and universal adapter.

Name		Sequence
miR-2861	Target	5’-GGG GCC UGG CGG UGG GCG G-3’
	Probe	/5ThioMC6-D/ GATATATTTTAC CGC CCA CCG CCA GGC CCC/3InvdT/
miR-486	Target	5’-UCC UGU ACU GAG CUG CCC CGA G-3’
	Probe	/5ThioMC6-D/ GATATATTTTAC TCG GGG CAG CTC AGT ACA GGA/3InvdT/
miR-6880	Target	5’-UGG UGG AGG AAG AGG GCA GCU C-3’
	Probe	/5ThioMC6-D/ GATATATTTTAG AGC TGC CCT CTT CCT CCA CCA/3InvdT/
Universal adapter		/5Phos/TAAAATATATAAAAAAAAAAAA/3Bio/

Table S2. Comparison of colorimetry-based miRNA detection platforms

	Limit of detection	Total assay time	Multiplexing capacity
Ag/Pt nanocluster-based assay[1]	18 pM	>30 min	1
DNAzyme-based assay[2]	480 pM	50 min	1
Gold nanoparticles-based assay [3]	50 pM	120 min	1
DSF particles-based assay[4]	4.5-23.7 pM	225 min	10^5
PSF particles-based assay (this work)	7-15 pM	135 min	10^5

Reference

1. Fakhri, Neda, et al. "Paper based colorimetric detection of miRNA-21 using Ag/Pt nanoclusters." *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 227 (2020): 117529.

2. Park, Yeonkyung, et al. "Universal, colorimetric microRNA detection strategy based on target-catalyzed toehold-mediated strand displacement reaction." *Nanotechnology* 29.8 (2018): 085501.

3. Liu, Pei, et al. "Enzyme-free colorimetric detection of DNA by using gold nanoparticles and hybridization chain reaction amplification." *Analytical chemistry* 85.16 (2013): 7689-7695.

4. Juthani, Nidhi, and Patrick S. Doyle. "A platform for multiplexed colorimetric microRNA detection using shape-

encoded hydrogel particles." *Analyst* 145.15 (2020): 5134-5140.