

Supporting Information for

A simple, sensitive, and label-free assay for alkaline phosphatase activity based on target-promoted exponential strand displacement amplification

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Table S1. Comparison of this method with previous fluorescence-based methods.

Key material/method	Detection limit (U/L)	Assay time	Limitations	Reference
Carbon quantum dot	1.4	1 hr 20 min	- Synthesis of nanomaterials - Low sensitivity	[1]
Enzymatic hydrogelation	60	3 hr	- Synthesis of organic probes - Low sensitivity - Signal-off	[2]
Stimulus-responsive infinite coordination polymer nanoparticles	10	real-time monitoring	- Synthesis of organic probes - Low sensitivity	[3]
DNA-templated silver nanoclusters	5	2 hr 45 min	- Low sensitivity - Signal-off	[4]
Cu(I)-catalyzed cycloaddition reaction and Cu(II)-dependent DNAzyme	0.14	4 hr	- Labeling with fluorophore and quencher	[5]
Cleavage by λ exonuclease and fluorescence quenching by graphene oxide	0.19	1 hr 10 min	- Synthesis of nanomaterials - Labeling with fluorophore	[6]
Elongation with hairpin primer	3	2 hr 25 min	- Synthesis of nanomaterials - Low sensitivity	[7]
eSDA	0.47	1 hr 20 min	-	This work

Table S2. DNA sequences employed in this work.

Strand name	DNA sequence (5' □ 3')
FP ^(a,b,c)	AAA AAA <u>AGG ATC</u> TAG TGC GTC TCG G-PO ₄
RP ^(a,b,c)	AAA AAA <u>AGG ATC</u> GCG GTC GGA AGC T-PO ₄
TP1 ^(b,c)	GCG GTC GGA AGC TCC TAT GAC AAT GCA CTA GCC GAG ACG CAC-PO ₄
FP-OH ^(a,b,c)	AAA AAA <u>AGG ATC</u> TAG TGC GTC TCG G
RP-OH ^(a,b,c)	AAA AAA <u>AGG ATC</u> GCG GTC GGA AGC T
TP1-OH ^(b,c)	GCG GTC GGA AGC TCC TAT GAC AAT GCA CTA GCC GAG ACG CAC

^(a) The recognition sequence for nicking endonuclease is underlined.

^(b) The sequence in FP complementary to TP1 is highlighted in blue, which is also applied to FP-OH and TP1-OH.

^(c) The sequence in RP identical to TP1 is highlighted in red, which is also applied to RP-OH and TP1-OH.

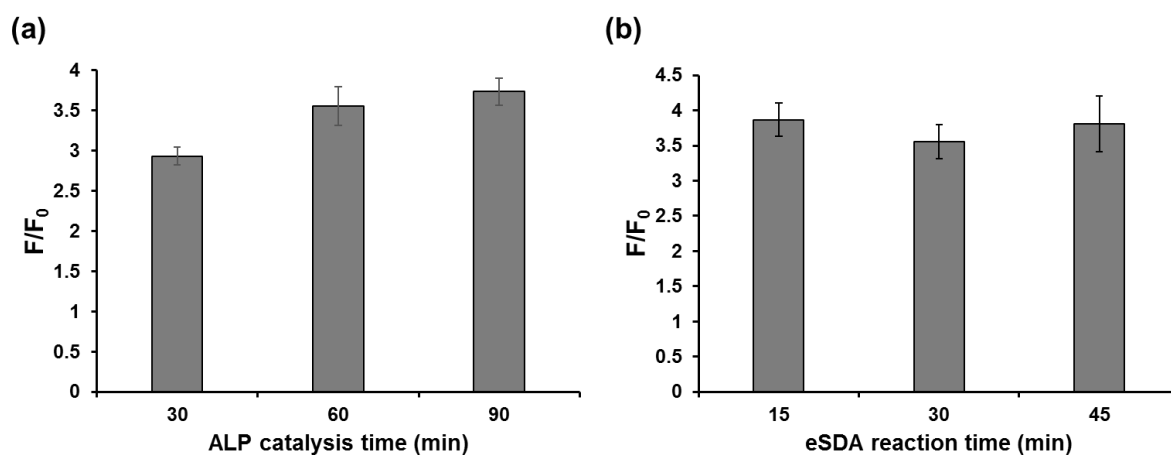


Fig. S1. Optimization of the reaction times for ALP catalysis and eSDA. (a) Fluorescence signal-to-background ratios (F/F_0) at different ALP catalysis times (eSDA reaction time: 30 min). (b) F/F_0 at different eSDA reaction times (ALP catalysis time: 60 min). F_0 and F are the fluorescence intensities at 525 nm from SYBR green I in the absence and presence of ALP, respectively. The final concentrations of KF, Nt.AlwI, SYBR green I, and ALP are 20 U/mL, 120 U/mL, 1X, and 1 U/mL, respectively.

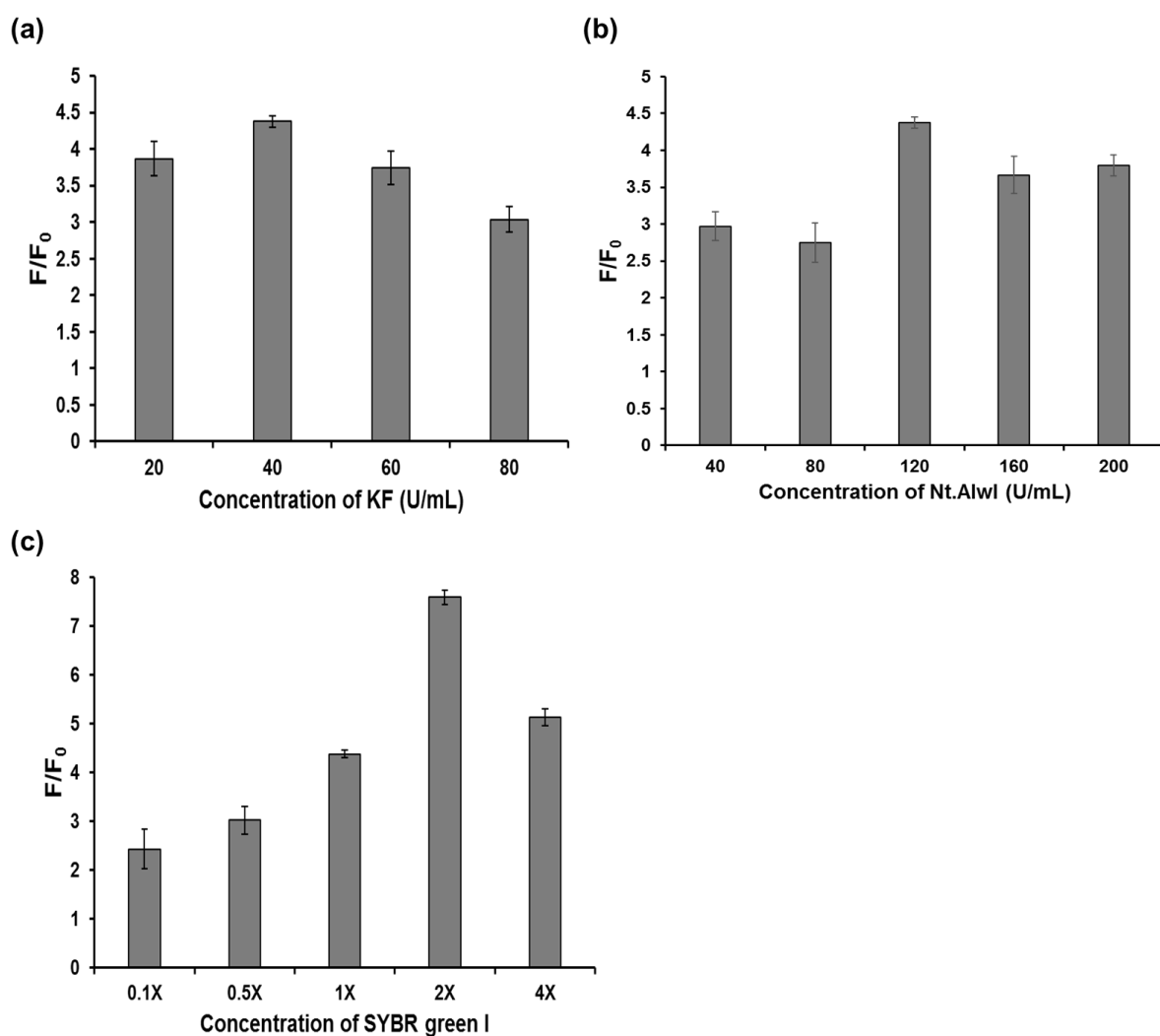


Fig. S2. Optimization of the reaction concentrations of KF, Nt.AlwI, and SYBR green I. (a) Fluorescence signal-to-background ratios (F/F_0) at different KF concentrations (Nt.AlwI concentration: 120 U/mL, SYBR green I concentration: 1X). (b) F/F_0 at different Nt.AlwI concentrations (KF concentration: 40 U/mL, SYBR green I concentration: 1X). (c) F/F_0 at different SYBR green I concentrations (KF concentration: 40 U/mL, Nt.AlwI concentration: 120 U/mL). F_0 and F are the fluorescence intensities at 525 nm from SYBR green I in the absence and presence of ALP, respectively. The final concentration of ALP is 1 U/mL.

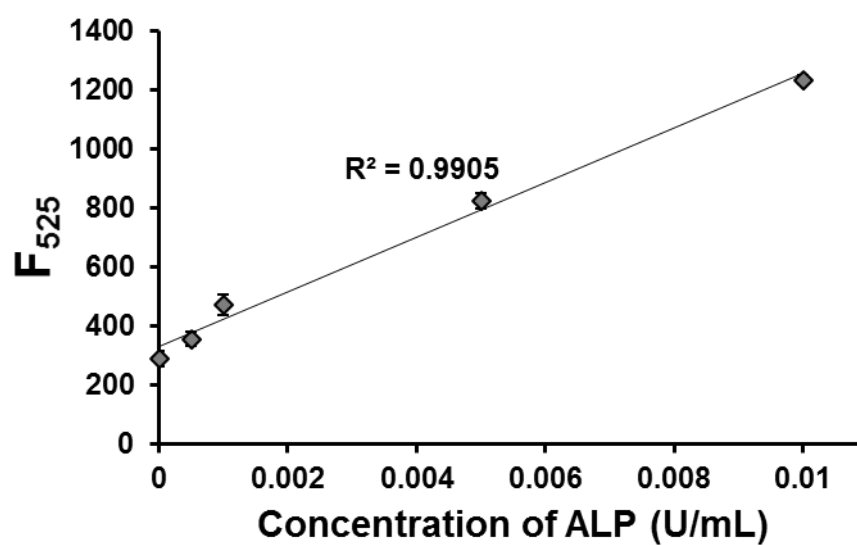


Fig. S3. The linear relationship between the fluorescence intensity at 525 nm from SYBR green I and the ALP activity spiked in diluted human serum (1%).

References

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