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Electronic Supplementary Information

Primer dephosphorylation-initiated circular exponential amplification for ultrasensitive detection of alkaline phosphatase

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Optimization of ALP-catalyzed dephosphorylation reaction time

ALP-catalyzed dephosphorylation of 3′-phosphoryl end of the primer is the most crucial step, and thus the reaction time of dephosphorylation should be optimized. As shown in Fig. S1, the fluorescence intensity increases with the reaction time, and reaches a plateau within 30 min. Thus, the reaction time of 30 min is used in subsequent experiments.

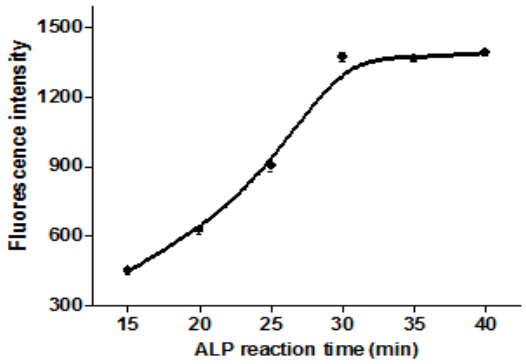

Fig. S1 Variance of fluorescence intensity with the dephosphorylation reaction time. Error bars show the standard deviation of three independent experiments.

Optimization of reaction temperature

We further optimized the reaction temperature of dual-functional hairpin probe-mediated circular exponential amplification. As shown in Fig. S2, the fluorescence intensity enhances when reaction temperature increases from 52 to 58 °C, but decreases beyond 58 °C. This can be explained by that (1) too high reaction temperature (>58 °C) may prevent the formation of hairpin structures for HP1 and HP2, adversely affecting the dual-functional hairpin probe-mediated circular exponential amplification, and (2) a high reaction temperature may inhibit the activity of Nt.BstNBI whose optimal reaction temperature is 55 °C. Because the maximum fluorescence intensity is obtained at the reaction temperature of 58 °C, the temperature of 58 °C is used in subsequent experiments.

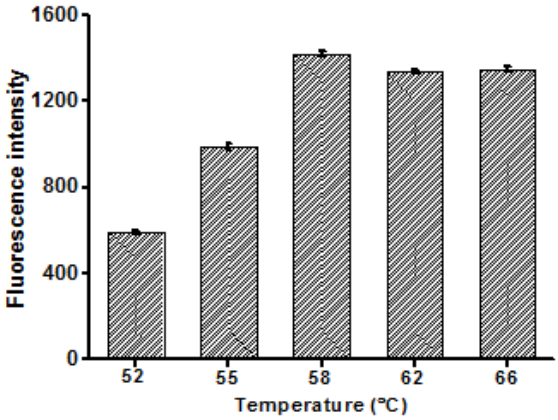

Fig. S2 Variance of fluorescence intensity with different reaction temperatures. Error bars show the standard deviations of three independent experiments.

Optimization of the ratio of HP1 to HP2

To investigate the effect of the HP1-to-HP2 ratio upon the amplification efficiency of the circular exponential amplification reaction, we monitored the variance of fluorescence intensity with different HP1-to-HP2 ratio. As shown in Fig. S3, the fluorescence intensity enhances with the increasing HP1-to-HP2 ratio from 3:1 to 1:3, and reaches the maximum value at the ratio of 1:3, followed by the decrease beyond the ratio of 1:3. Thus, the HP1-to-HP2 ratio of 1:3 is used in the subsequent experiments.

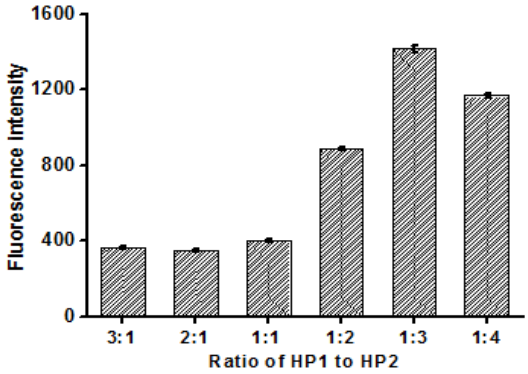

Fig. S3 Variance of fluorescence intensity with different ratio of HP1 to HP2. Error bars show the standard deviations of three independent experiments.

Table S1. Comparison of the proposed method with the reported methods for ALP assay

strategy	signal on/off	assay time*	detection limit (U/μL)	cell analysis	reference
fluorescent assay based on EXPAR [a]	signal on	~ 1.7 h	2.0×10^{-10}	yes	this work
fluorescent assay based on DNA-scaffolded silver nanocluster	signal off	~ 3.3 h	5×10^{-6}	no	1
fluorescent assay based on enzymatic hydrogelation	signal off	Over 24 h	6×10^{-5}	yes	2
fluorescent assay based on inner filter effect	signal off	Over 2 days	1.0×10^{-9}	yes	3
fluorescent assay based on photo-induced electron transfer	signal off	over 4 days	9×10^{-7}	no	4
ratiometric fluorescent assay based on electrospun fibrous strips	signal on	over 2 days	1.5×10^{-6}	no	5
colorimetric assay based on guanine-rich DNAzyme	signal on	~ 2 h	8.4×10^{-7}	no	6
colorimetric assay based on the conjugated AuNP/ATP [b]	signal on	over 2 days	1×10^{-7}	no	7
ratiometric electrochemical	signal on	over 24 h	4×10^{-7}	no	8

assay based on ferrocene-derived substrate					

[a] EXPAR, exponential amplification reaction; [b] AuNP/ATP, gold nanoparticle/adenosine triphosphate. * Assay time includes the preparation time.

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