Electronic Supplementary Information

pH-based immunoassay: explosive generation of hydrogen ions through an immuno-triggered nucleic acid exponential amplification reaction

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Table S1. Sequences of oligonucleotides.

Oligonucleotides	**Sequences (5'-3')**	**Modification**
Template	GCATCTACCTCAGCATCTACCTCA	3'-C3 Spacer
Primer	TGAGGTAGATGC▽TGAGGTAGATGC	
Biotin-primer	TGAGGTAGATGC▽TGAGGTAGATGC	5'-biotin
Biotin-m-primer	GAAGCTGTTGTAATATCACTGAAA	5'-biotin

Note: The red marked bases are the recognition sequence of nicking enzyme and "▽" represents the nicking site.

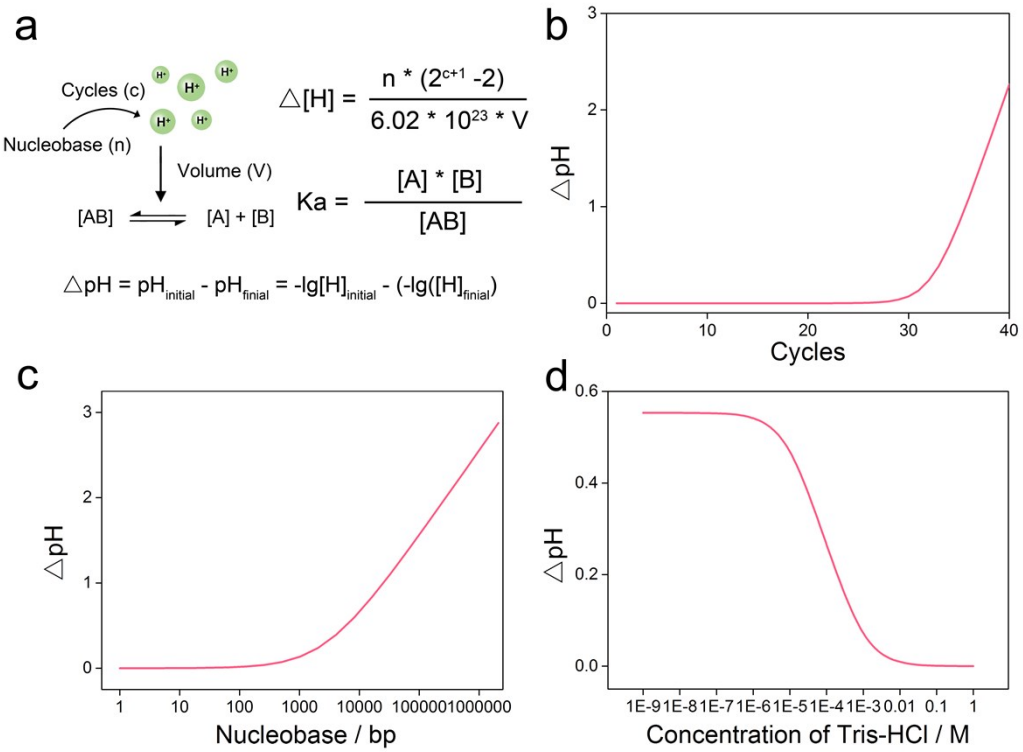

Fig. S1 The influence of some factors on pH changes. (a) Formula for calculating the changes of pH. (b) The influence of cycle numbers on the changes of pH (nucleobase = 500 bp, template = 1, volume = 100 μL, initial pH = 7). (c) The influence of nucleobase numbers on the changes of pH (template = 1, cycles = 30, volume = 100 μL, initial pH = 7). (d) The influence of the concentration of Tris-HCl on the changes of pH (template = 1, cycles = 37, nucleobase = 500 bp, volume = 100 μL, initial pH = 8.2, (Effective buffering range of Tris-HCl: 7.0 - 9.2, Ka = $10^{-8.1}$)).

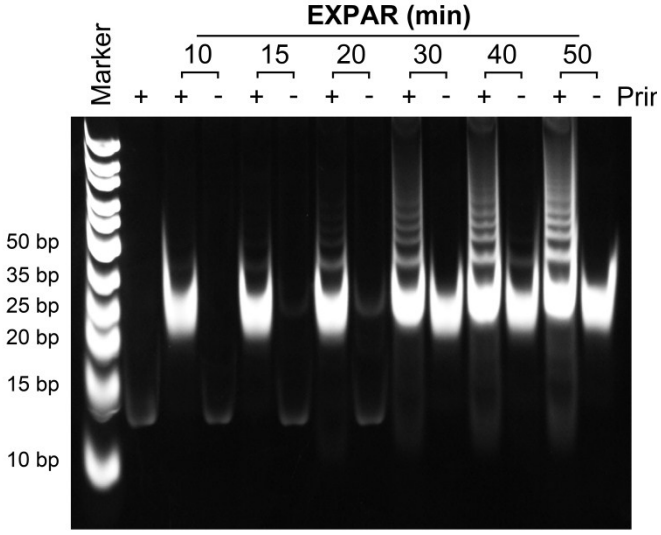

Fig. S2 Polyacrylamide gel electrophoretic patterns of EXPAR products with different amplification times.

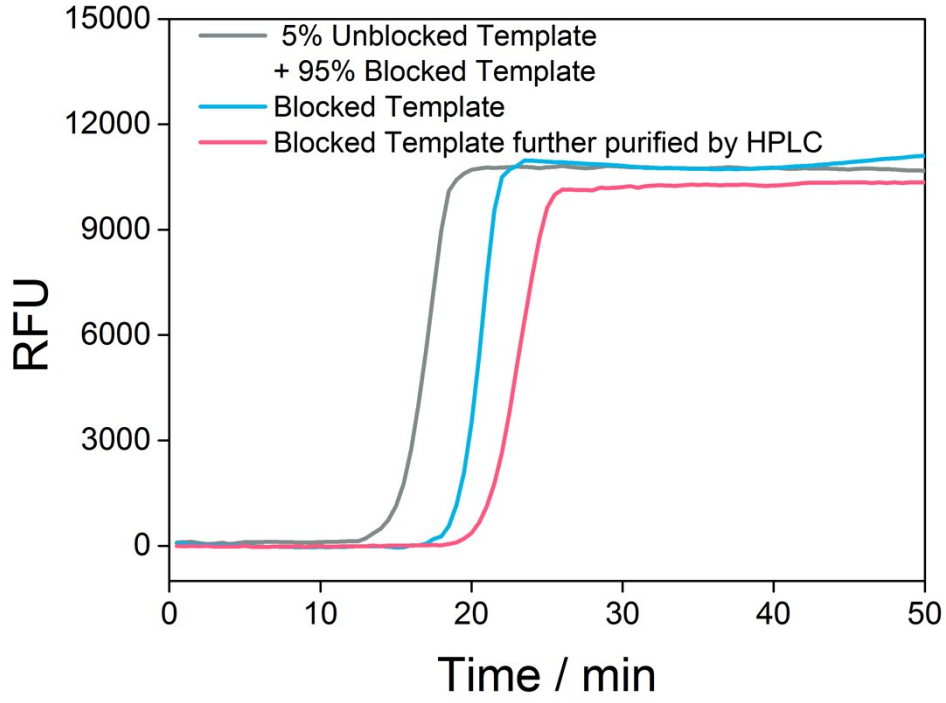

Fig. S3 The influence of the purification of C3-Spacer blocked template on unspecific amplification.

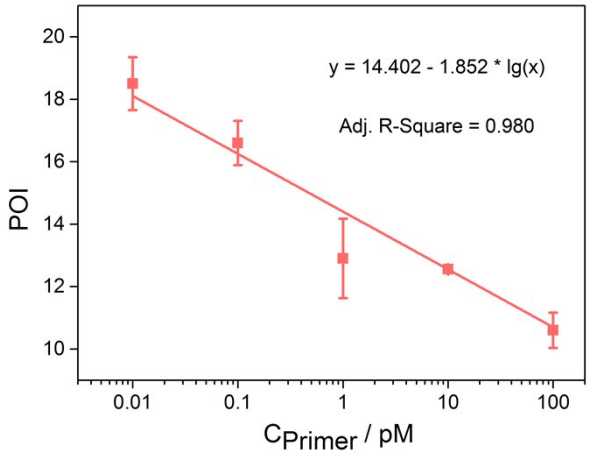

Fig. S4 Log-linear relationship between the concentration of primer ranging from 10 fM to 100 pM and the point of inflection (POI) obtained from the quantification real-time EXPAR (Fig. 1f). (linear regression equation: $y=14.402-1.852x$, $R^2=0.980$).

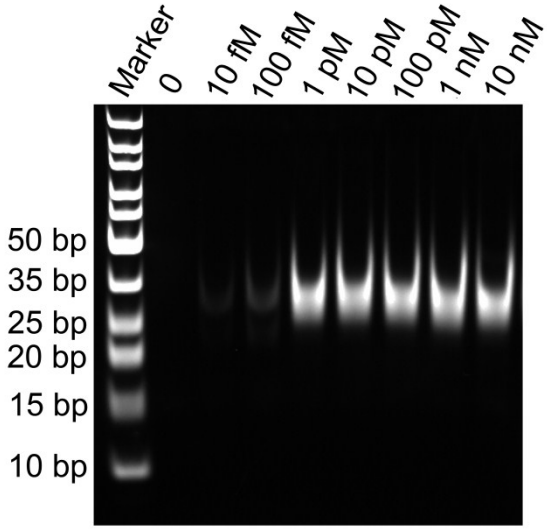

Fig. S5 Polyacrylamide gel electrophoretic patterns of EXPAR products with different primer concentrations.

Table S2. Determination of spiked human PDGF-BB in 10% serum samples.

Sample	Added PDGF-BB (ng/L)	Found PDGF-BB (ng/L)	Recovery (%)	R.S.D. (%, n=3)
1	20	18.5	93	3
2	4	3.89	97	3
3	0.3	0.311	104	3

Table S3. Determination of spiked CAP in 10% serum samples.

Sample	Added CAP (nM)	Found CAP (nM)	Recovery (%)	R.S.D. (%, n=3)
1	3.71	3.34	90	5
2	0.464	0.432	93	2
3	0.0309	0.0315	102	1