SUPPLEMENTARY INFORMATION

Detection of thrombin based on aptamer isothermal exponential signal amplification technique

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Supplementary Figures

Table 1. Sequences of molecular switch and primers

	Sequences (5’ to 3’)
Hairpin 1 (H1)	5’-CTCATCATAG**GTTCTGTAG**GGTTG GTGTGGTTGG**ACTAAGGTTA**CTAC AGAACAT-3’
Primer 1 (P1)	5’-*CGGATCTTCT*ATGTTCTGTA-3’
Primer 2 (P2)	5’-*CGGATCTTCT*CTCATCATAG-3’

The 5'-end of H1 is region 1, the 10 free bases is toehold for the second cycle. The boldfaced portion is region 2, indicates the stem of H1 which composed by 9 complementary bases. The shaded portion in H1 is region 3, indicates the loop of H1 which contained thrombin aptamer sequences (the character borders). The emphasis character is region 4, indicates the other stem of H1, complete complementary with region 2. The underlined portion is region 5, the opened H1 can hybrided with P2 to form the duplex is the nicking site of the nicking enzyme *Nt.Alw*I.

In the two primers, the italic portion is the nicking site of the nicking enzyme *Nt.Alw*I, the underlined portion is the template to form the duplex.

Fig. S1. The optimum of the concentration ratio of two primers. The 10 μL reaction containing 100 fmol thrombin, 1.0×10^{-8} M H1, Primer 1, Primer 2, 1.5×10^{-4} M dNTPs, 0.5 U Klenow (exo^-) DNA polymerase, 6 U *Nt.Alw*I nicking enzyme, 0.25 μL 20×SYBR Green I, and 1×CutSmart buffer.

1~3 were P2:P1 = 50:1, 1:1 and 100:1, **4~6** were the negative controls of **1~3**, respectively. The reactions were amplified for 40 min at 37℃.

Fig. S2. The optimum of incubation time. The 10 μL reaction containing 100 fmol thrombin, 1.0×10^{-8} M H1, 1.0×10^{-8} M P1, 5.0×10^{-7} M P2, 1.5×10^{-4} M dNTPs, 0.5 U Klenow (exo^-) DNA polymerase, 6 U *Nt.Alw*I nicking enzyme, 0.25 μL 20×SYBR Green I, and 1×CutSmart buffer.

1. 10 min, 2**.** 20 min and **3.** 30 min. **4, 5** and **6** were the negative controls of **1~3**, respectively. The reactions were amplified for 40 min at 37℃.

Fig. S3. The optimum of reaction temperature. The 10 μL reaction containing 100 fmol thrombin, 1.0×10^{-8} M H1, 1.0×10^{-8} M P1, 5.0×10^{-7} M P2, 1.5×10^{-4} M dNTPs, 0.5 U Klenow (exo^-) DNA polymerase, 6 U *Nt.Alw*I nicking enzyme, 0.25 μL 20×SYBR Green I, and 1×CutSmart buffer.

1. 31 ℃, **2.** 33 ℃, **3.** 35 ℃, **4**. 37 ℃ and **5**. 39 ℃. **6~10** were the negative controls respectively. The reactions were incubated for 20 min at 37℃.