Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Flap endonuclease-initiated enzymatic repairing amplification for ultrasensitive detection of target nucleic acids

Hyowon Jang[#a], Chang Yeol Lee[#a], Seoyoung Lee[a], Ki Soo Park[b*] and Hyun Gyu Park[a*]

[a] Department of Chemical and Biomolecular Engineering (BK 21+ program),
KAIST, Daehak-ro 291, Yuseong-gu, Daejeon 305-701, Republic of Korea

[b] Department of Biological Engineering, College of Engineering, Konkuk University, Seoul 05029, Republic of Korea.

#These authors equally contributed to this work.

* To whom correspondence should be addressed.

E-mail: hgpark@kaist.ac.kr (H.G. Park); Phone: +82-42-350-3932; Fax: +82-42-350-3910.

E-mail: kskonkuk@gmail.com (K.S. Park); Phone: +82-2-450-3742; Fax: +82-2-450-3742

Table S1 DNA sequences employed in this work.

Strand name	**DNA sequence (5' → 3')[a]**
Target specific-primer[b]	GTC TGA CGG TTC TTA AGC T**C**
FERA primer[c]	**AAA ACA CAG ACA GGA AAA GAA G G**GG AGA AAG AAA TGG TAG CAA CA-phosphate
FERA template	**CCT TCT TTT CCT GTC TGT GTA CTC CTC GGC GCT A CC TTC TTT TCC TGT CTG TGT TTT A TA GTC TGT TTC TGT GGT GT**
FERA invasive probe	**ACA CCA CAG AAA CAG ACT A**CC CGT CTC GGT TTT CCG AGA CGG GTT **CCT CGG CGC T**
Target DNA	CAA CAA GCT ACC ATT TCT TTC TCC C[d]AG CTT AAG AAC CGT CAG ACA GAA AA
T1	CAA CAA GCT ACC ATT TCT TTC TCC T[d]AG CTT AAG AAC CGT CAG ACA GAA AA
T2	CAA CAA GCT ACC ATT TCT TTC TCC G[d]AG CTT AAG AAC CGT CAG ACA GAA AA
T3	CAA CAA GCT ACC ATT TCT TTC TCC A[d]AG CTT AAG AAC CGT CAG ACA GAA AA
Asymmetric PCR forward primer for CT plasmid DNA	AGA GAA CGT GCG GGC G
Asymmetric PCR reverse primer for CT plasmid DNA	TTA GTC AGA TTT GTT TCC AAC AAG CTA

(a) The colors of oligonucleotide sequences correspond to those of the domains depicted in Figure 1.

(b) The target-specific primer is designed to be complementary to the target nucleic acids (black) except for one base at 3'-end (red) that is overlapped with one base of the FERA primer to form the junction of the single- and double-stranded DNA that can be cleaved by FEN.

(c) The FERA primer is designed to contain trigger 1 (green) and the sequence complementary to the target nucleic acids (black) with the 3'-phosphopate group (red).

(d) The underlined bases in T1, T2, and T3 indicate the mismatched site that is different from target DNA.

Table S2 Comparison of this method with other isothermal amplification methods.

Key method	Signaling	Assay time (min)	Detection limit	Limitations	Reference
Self-primed isothermal amplification	Electrochemistry	90	0.1 pM	-Labeling with thiol -Requirement of nicking enzyme recognition site in target nucleic acid	1
Hybridization chain reaction coupled with SiO_2@$MoSe_2$[a] and GO-AuNPs[b]	Electrochemistry	265	0.068 fM	-Long reaction time -Modification of electrode	2
SDA[c] and hairpin self-assembly	Electrochemistry	160	1.6 fM	- Labeling with electrochemical active molecule - Modification of electrode	3
EXPAR-assisted AuNP[d] amplification	Colorimetry	60	46 fM	Poor stability of AuNPs[d] in enzymatic reaction buffers	4
Catalytic hairpin assembly coupled with enzymatic repairing amplification	Fluorescence	120	50 fM	Labeling with fluorophore and quencher	5
Cyclic enzymatic repairing amplification	Fluorescence	130	0.1 fM	Labeling with fluorophore and quencher	6
FERA	Fluorescence	90	15.16 aM	-	This study

[a] SiO_2@$MoSe_2$: silicon dioxide/molybdenum selenide

[b] GO-AuNPs: graphene oxide-gold nanoparticles

[c] SDA: strand displacement amplification

[d] AuNPs: gold nanoparticles.

Fig. S1 The flowchart that shows the step-by-step procedures of the FERA assay.

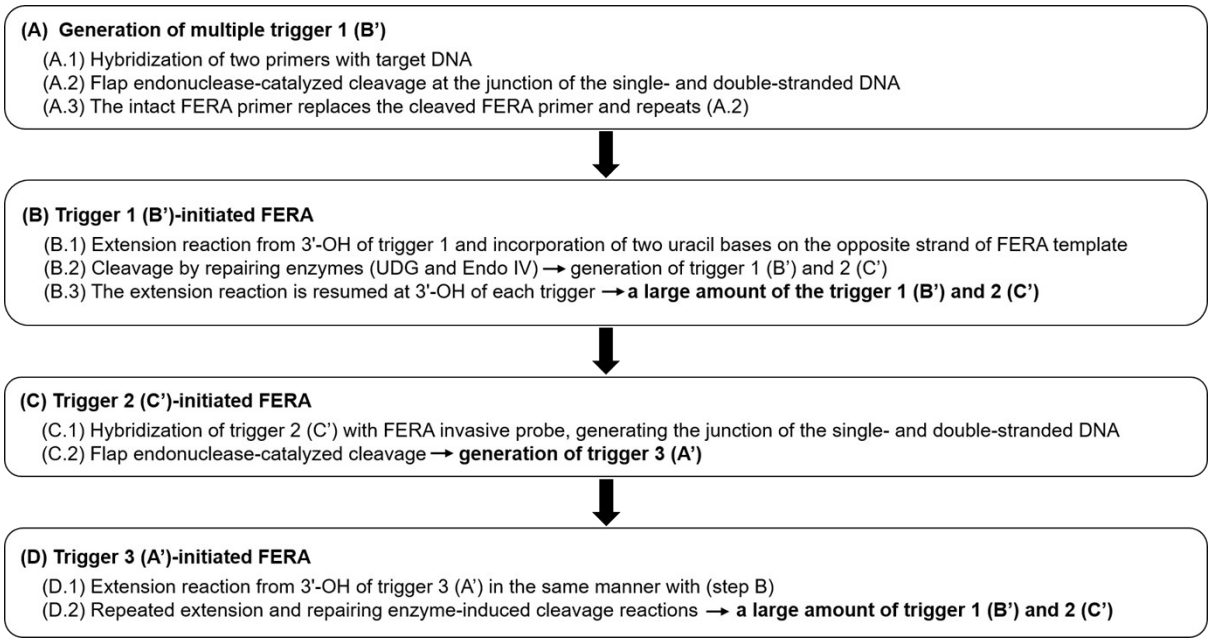


Fig. S2 Time-dependent fluorescence intensities during the FERA reaction to find the optimal 3'-end structure of the FERA primer (FERA primers without (A) and with (B) the phosphate group at 3'-end). The concentrations of target specific-primer, FERA primer, template, invasive probe, and target DNA are 5 nM, 5 nM, 50 nM, 500 pM, and 1 nM, respectively.

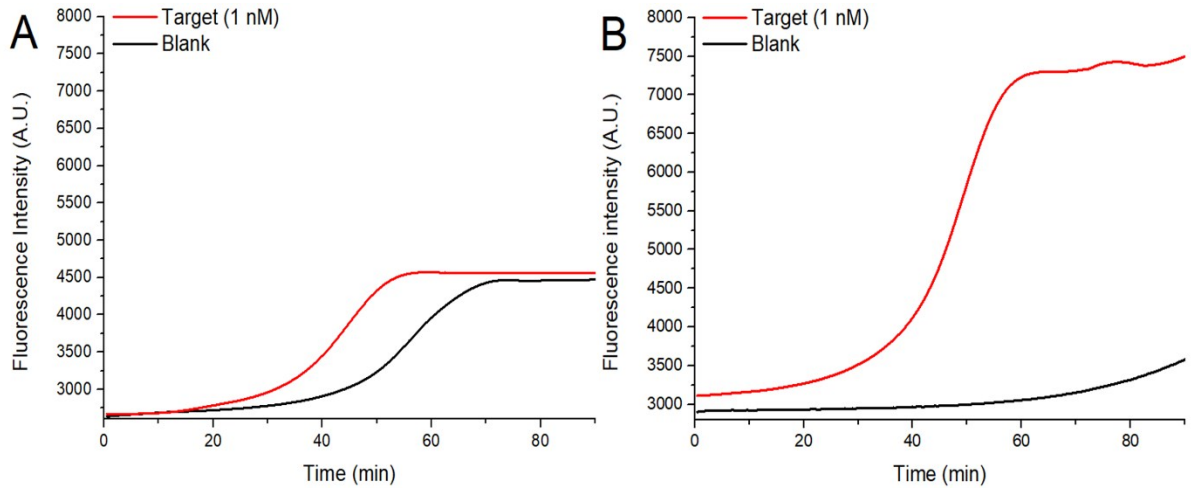


Fig. S3 Time-dependent fluorescence intensities during the FERA reaction to find the optimal reaction temperature. (A: 40 ℃, B: 42.5 ℃, C: 45 ℃, D: 47.5 ℃, E: 50 ℃). The concentrations of target specific-primer, FERA primer, template, invasive probe, and target DNA are 5 nM, 5 nM, 50 nM, 500 pM, and 1 nM, respectively.

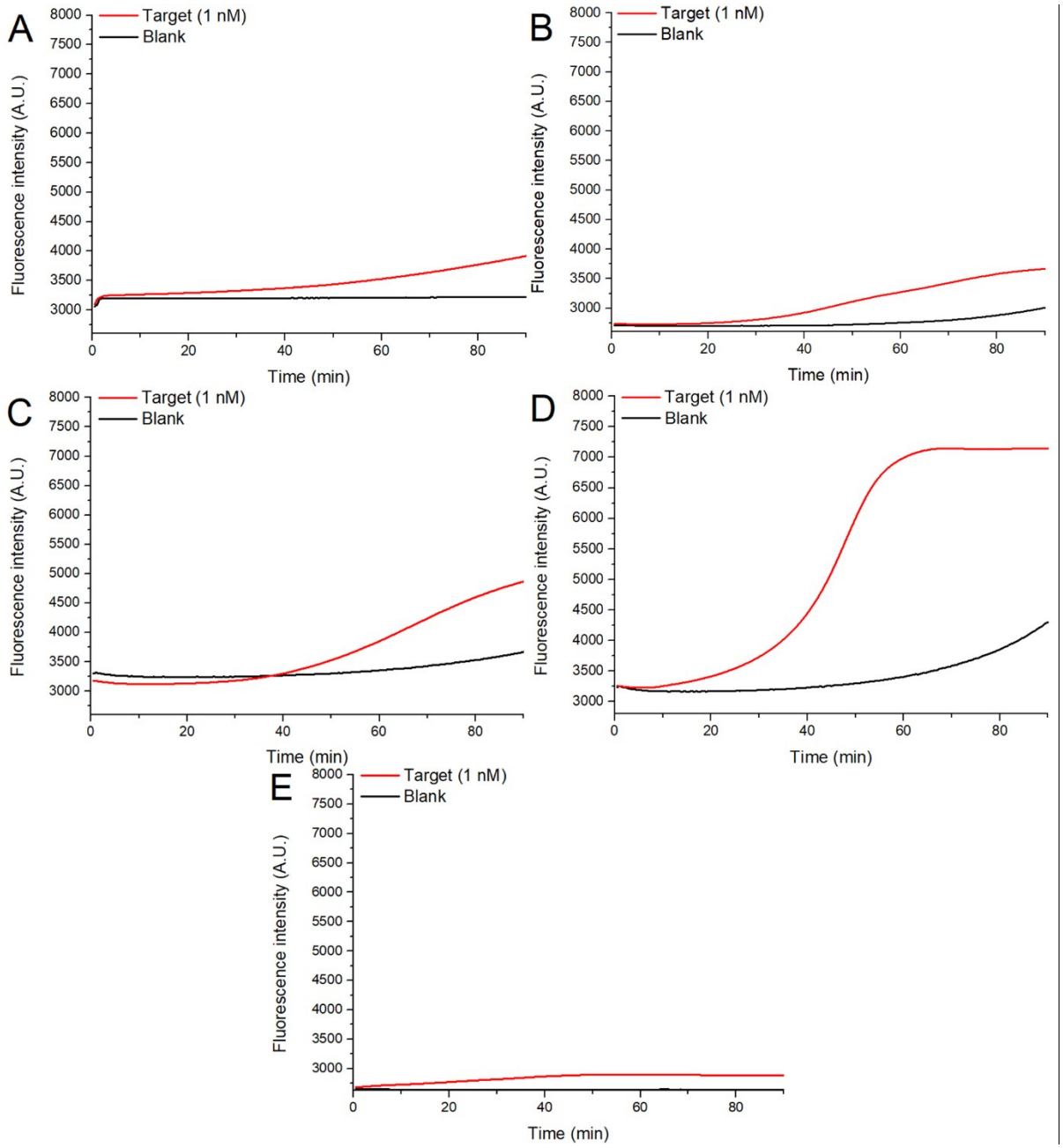


Fig. S4 Time-dependent fluorescence intensities during the FERA reaction to find the optimal concentration of FERA template (A: 250 nM, B: 100 nM, C: 50 nM, D: 10 nM, E: 5 nM). The concentrations of target specific-primer, FERA primer, invasive probe, and target DNA are 5 nM, 5 nM, 500 pM, and 1 nM, respectively.

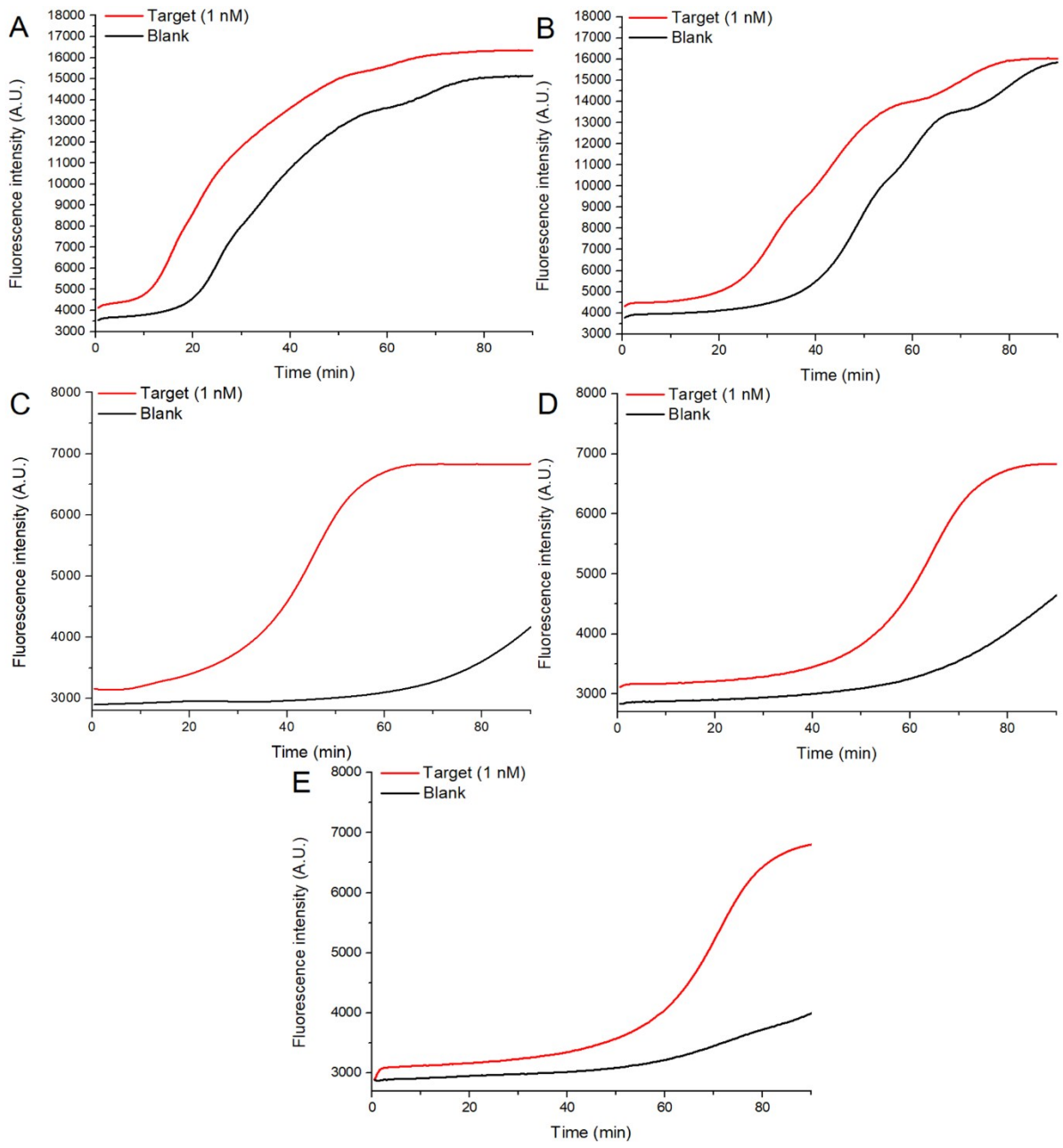


Fig. S5 Time-dependent fluorescence intensities during the FERA reaction to find the optimal concentration of FERA primer (A: 50 nM, B: 25 nM, C: 5 nM, D: 2.5 nM, E: 1 nM). The concentrations of target specific-primer, FERA template, invasive probe, and target DNA are 5 nM, 50 nM, 500 pM, and 1 nM, respectively.

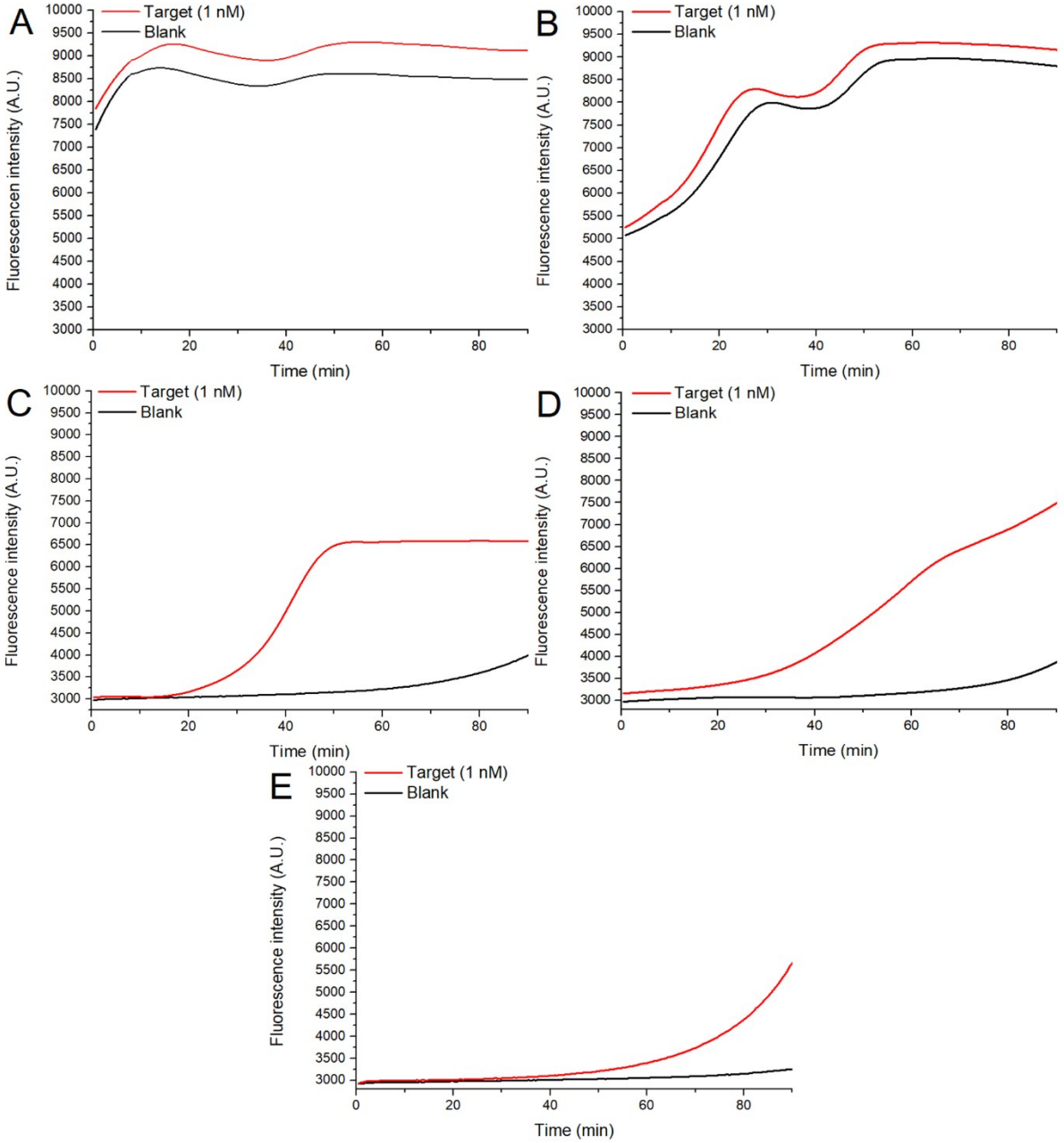


Fig. S6 Time-dependent fluorescence intensities during the FERA reaction to find the optimal concentration of FERA invasive probe (A: 10 nM, B: 5 nM, C: 1 nM, D: 500 pM, E: 100 pM). The concentrations of target specific-primer, FERA primer, template, and target DNA are 5 nM, 5 nM, 50 nM, and 1 nM, respectively.

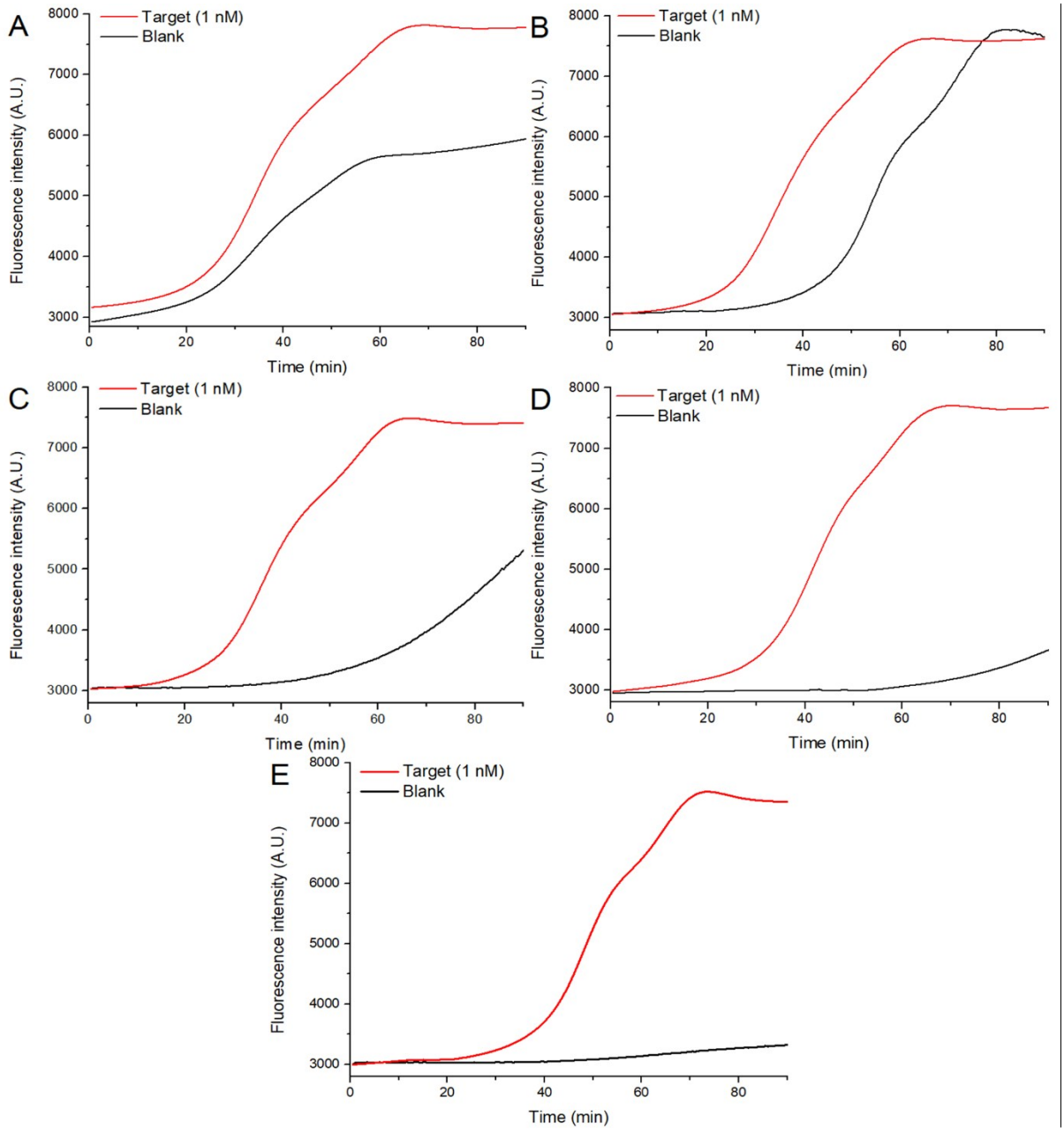
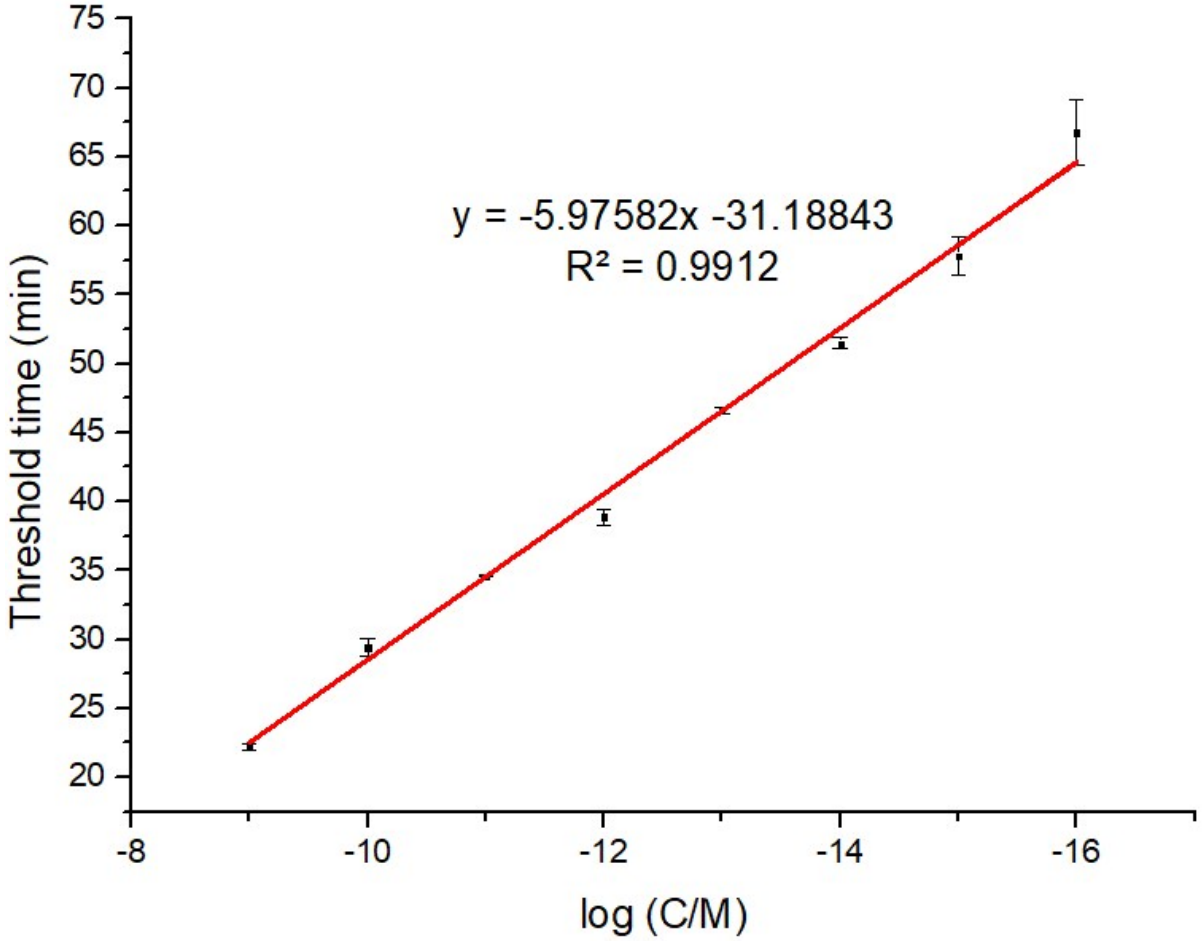


Fig. S7 The correlation of threshold time to logarithm of the concentration of asymmetric PCR products spiked in diluted human serum (1%). Threshold time was defined as the reaction time when the fluorescence intensity is over 3,400. The concentrations of target specific-primer, FERA primer, template, and invasive probe are 5 nM, 5 nM, 50 nM, and 500 pM, respectively. Error bars were obtained from the three repeated experiments.



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