Supplementary Information

Real-time electrochemical monitoring of isothermal helicase-dependent amplification of nucleic acids

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Real-time fluorescent-based PCR

Figure S1 shows the real-time PCR amplification curves for serial dilutions (10-fold) of the 89-bp target DNA sequence of E. Coli. The experimental conditions are described in the Experimental Section. Graph A in Figure S1 corresponds to the baseline corrected relative fluorescence intensity (ΔRn) of EvaGreen® dye versus amplification time, while graph B is the corresponding semi-logarithmic representation. The horizontal dashed line in graph B was used to determine the threshold cycle numbers that were next used to establish the calibration plot in C. A linear dynamic range between \(2 \times 10^3\) to \(5 \times 10^9\) initial target copies was determined from graph C, with a detection limit of \(~10^3\) initial target copies, while a PCR efficiency of 1.8 can be calculated from the slope, which is closed to the theoretical value of 2.
Figure S1. (A, B) EvaGreen®-based Real-time PCR amplification curves of the 89-bp DNA target. The concentration of primers in each reaction solution was 75 nM and the initial number of target DNA copies per assay was: (1) $5 \times 10^9$; (2) $5 \times 10^8$; (3) $5 \times 10^7$; (4) $5 \times 10^6$; (5) $5 \times 10^5$; (6) $5 \times 10^4$; (7) $5 \times 10^3$; (8) $5 \times 10^2$ and (9) 0 (NTC). (B) Semi-logarithmic representation of the relative fluorescence intensity as a function of PCR cycle number. (C) Calibration plot (slope: -3.96, intercept: 46.7, $r = 0.9995$).