Ultrasensitive detection of ALP based on utilizing self-priming template EXPAR reaction assay

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Herein, we describe an alkaline phosphatase (ALP) assay based on target-activated self-priming exponential isothermal amplification reaction. As a substrate of the ALP, this strategy utilizes single DNA probe, dubbed as a self-priming EXPAR template (SPT) with phosphate modification at its 3' end. The self-priming structure enables polymerase extension without additional primers, which significantly reduce the background signal and accelerate the reaction. The SPT is dephosphorylated by the ALP activity and then extended by the DNA polymerase. The extended SPT contains nicking enzyme cleavage site where repetitive nicking reaction and polymerase extension are performed. The DNA polymerase generates a lot of free short triggers that binds to the free SPTs to initiate further amplification reactions. As a result, double-stranded SPTs (dsSPT) are synthesized under isothermal condition, which can be monitored by the fluorescence from SYBR Green Ⅰ. We successfully detected ALP activity down to 0.00013 U/mL with high selectivity. The practical applicability of the developed strategy was also verified by applying the strategy for inhibitor screening.