Electronic Supplementary Material (ESI) for Analyst.

Phosphorothioated and Phosphate-Terminal Dumbbell (PP-TD) Probe-Based Rapid Detection of Polynucleotide Kinase Activity

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Table S1. Sequence information in this study

Name	Sequences
PP-TD Probe	5'-AAGAATTCTTAAGAATTCTTACATCGTTACGTTAGTGGAAAACCACTAAC-3'P
P-TD Probe	5'-AAGAATTCTTAAGAATTCTTACATCGTTACGTTAGTGGAAAACCACTAAC-3'OH
Loop-6	5'-AAGAATTCTTAAGAATTCTTACATCGTTACGTTAGTGGAAAAAACCACTAAC-3'P
Loop-8	5'-AAGAATTCTTAAGAATTCTTACATCGTTACGTTAGTGGAAAAAAAACCACTAAC-3'P
Bridge-8	5'-AAGAATTCTTAAGAATTCTTATCGTTACGTTAGTGGAAAACCACTAAC-3'P
Bridge-12	5'-AAGAATTCTTAAGAATTCTTAAACATCGTTACGTTAGTGGAAAACCACTAAC-3'P

* Underlined bases are phosphorothioate modified.

Table S2. Comparison with other reported fluorescence methods

Reference	Time (min)	Linear Range (U/μL)	LOD (U/μL)
(Li, Xu et al. 2017)	335	1.0×10^{-7} to 1.0×10^{-3}	4.36×10^{-8}
(Zhao, Liu et al. 2017)	280	5.0×10^{-4} to 5.0×10^{-2}	2.0×10^{-4}
(Feng, Wang et al. 2018)	130	1.0×10^{-5} to 3.0×10^{-4}	6.7×10^{-6}
(Chen, Wang et al. 2019)	185	1.0×10^{-6} to 1.0×10^{-5}	7.9×10^{-7}
(Jie, Li et al. 2019)	250	1.0×10^{-6} to 1.0×10^{-2}	1.0×10^{-6}
(Li, Ma et al. 2019)	60	1.0×10^{-5} to 1.25×10^{-2}	1.0×10^{-5}
(Zhang, Liu et al. 2019)	190	7.0×10^{-5} to 1.5×10^{-2}	6.0×10^{-5}
(Zhou, Tong et al. 2019)	200	1.0×10^{-3} to 1.0×10^{-1}	2.0×10^{-4}
(Wang, Wang et al. 2020)	80	1.0×10^{-8} to 2.5×10^{-5}	3.3×10^{-9}
(Wu, He et al. 2020)	30	2.5×10^{-4} to 1.0×10^{-3}	1.1×10^{-4}
(Zhang, Fan et al. 2021)	180	5.0×10^{-10} to 5.0×10^{-6}	1.2×10^{-10}
This work	105	1.0×10^{-6} to 5.0×10^{-5}	7.7×10^{-7}

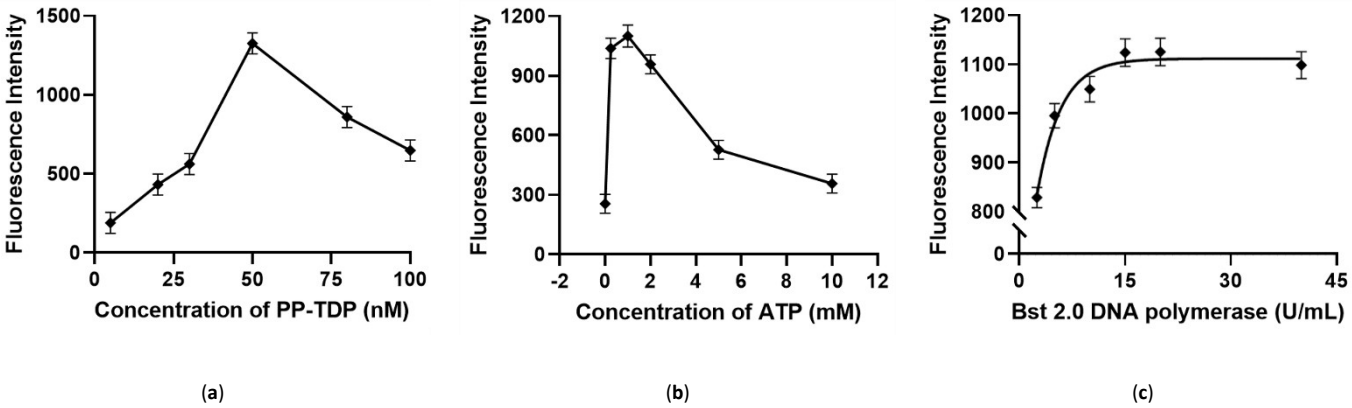

Figure S1. Optimization of the reaction conditions. (**a**) Fluorescence intensity vs. Concentration of PP-TD probe; (**b**) Fluorescence intensity vs. Concentration of ATP; (**c**) Fluorescence intensity vs. Concentration of Bst 2.0 DNA polymerase. The statistical data is presented as mean ± S.D (n=3).

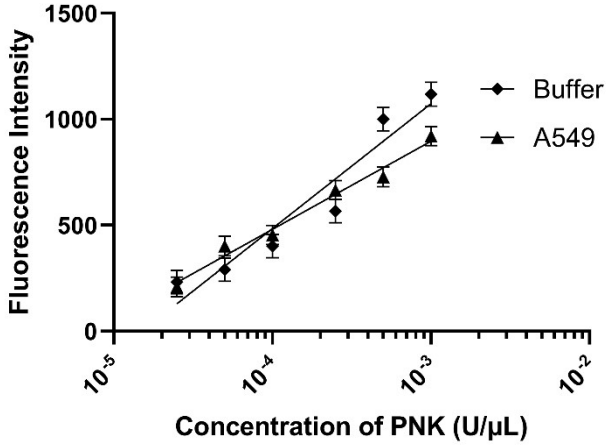

Figure S2. Quantitative detection in diluted cell extracts. The statistical data is presented as mean ± S.D (n=3).

References

1 Chen, H., Z. Wang, X. Chen, K. Lou, A. Sheng, T. Chen, G. Chen and J. Zhang (2019). "New method for detection of T4 polynucleotide kinase phosphatase activity through isothermal EXPonential amplification reaction." Analyst 144(6): 1955-1959.

2 Feng, C., Z. Wang, T. Chen, X. Chen, D. Mao, J. Zhao and G. Li (2018). "A Dual-Enzyme-Assisted Three-Dimensional DNA Walking Machine Using T4 Polynucleotide Kinase as Activators and Application in Polynucleotide Kinase Assays (vol 90, pg 2810, 2018)." Analytical Chemistry 90(7): 4932-4932.

3 Jie, G., C. Li, Y. Zhao, Q. Kuang and S. Niu (2019). "Fluorescent Mn: ZnCdS@ZnS and CdTe Quantum Dots Probes on 5102 Microspheres for Versatile Detection of Carcinoembryonic Antigen and Monitoring T4 Polynucleotide Kinase Activity." ACS Applied Nano Materials 2(7): 4637−4645.

4 Li, J., J. Ma, Y. Zhang, Z. Zhang and G. He (2019). "A fluorometric method for determination of the activity of T4 polynucleotide kinase by using a DNA-templated silver nanocluster probe." Microchimica Acta 186(1): 48.

5 Li, X., X. Xu, J. Song, Q. Xue, C. Li and W. Jiang (2017). "Sensitive detection of T4 polynucleotide kinase activity based on multifunctional magnetic probes and polymerization nicking reactions mediated hyperbranched rolling circle amplification." Biosensors and Bioelectronics 91: 631-636.

6 Wang, D.-X., J. Wang, Y.-C. Du, J.-Y. Ma, S.-Y. Wang, A.-N. Tang and D.-M. Kong (2020). "CRISPR/Cas12a-based dual amplified biosensing system for sensitive and rapid detection of polynucleotide kinase/phosphatase." Biosensors and Bioelectronics 168: 112556.
7 Wu, X., S. He and J. X. Zhao (2020). "Label-free fluorescence assay coupled exonuclease reaction and SYBR Green I for the detection of T4 polynucleotide kinase activity." Analytical Methods 12(6): 807-812.
8 Zhang, L., W. Fan, D. Jia, Q. Feng, W. Ren and C. Liu (2021). "Microchamber-Free Digital Flow Cytometric Analysis of T4 Polynucleotide Kinase Phosphatase Based on Single-Enzyme-to-Single-Bead Space-Confined Reaction." Analytical Chemistry 93(44): 14828-14836.
9 Zhang, X., Q. Liu, Y. Jin and B. Li (2019). "Determination of the activity of T4 polynucleotide kinase phosphatase by exploiting the sequence-dependent fluorescence of DNA-templated copper nanoclusters." Microchimica Acta 186(1): 3.
10 Zhao, H., Q. Liu, M. Liu, Y. Jin and B. Li (2017). "Label-free fluorescent assay of T4 polynucleotide kinase phosphatase activity based on G-quadruplexe-thioflavin T complex." Talanta 165: 653-658.
11 Zhou, H., C. Tong, W. Zou, Y. Yang, Y. Liu, B. Li, Y. Qin, W. Dang, B. Liu and W. Wang (2019). "A novel fluorescence method for activity assay and drug screening of T4 PNK by coupling rGO with ligase reaction." Analyst 144(4): 1187-1196.