# Electronic Supplementary Information (ESI)

# Amplified Fluorescence Detection of T4 Polynucleotide Kinase Activity and Inhibition via Coupled λ Exonuclease Reaction and Exonuclease III-aided Trigger DNA Recycling

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**Table S1**. Oligonucleotide sequences used in this work

| Name | Sequences |
|---|---|
| tDNA | 5’-C3-GGCTCTGACAACAAGATTACTAGTAGCTTTT-3’ |
| cDNA | 5’-GCTACTAGTAATCTTGTTGTCAGAGCCTTTT-3’ |
| P1-FAM | 5’-(FAM)-GACAACAAGATTACTAGTAGCTTTT-3’ |
| P2-Dabcyl | 5’-GCTACTAGTAATCTTGTTGT-(Dabcyl)-CAGAGCC-3’ |

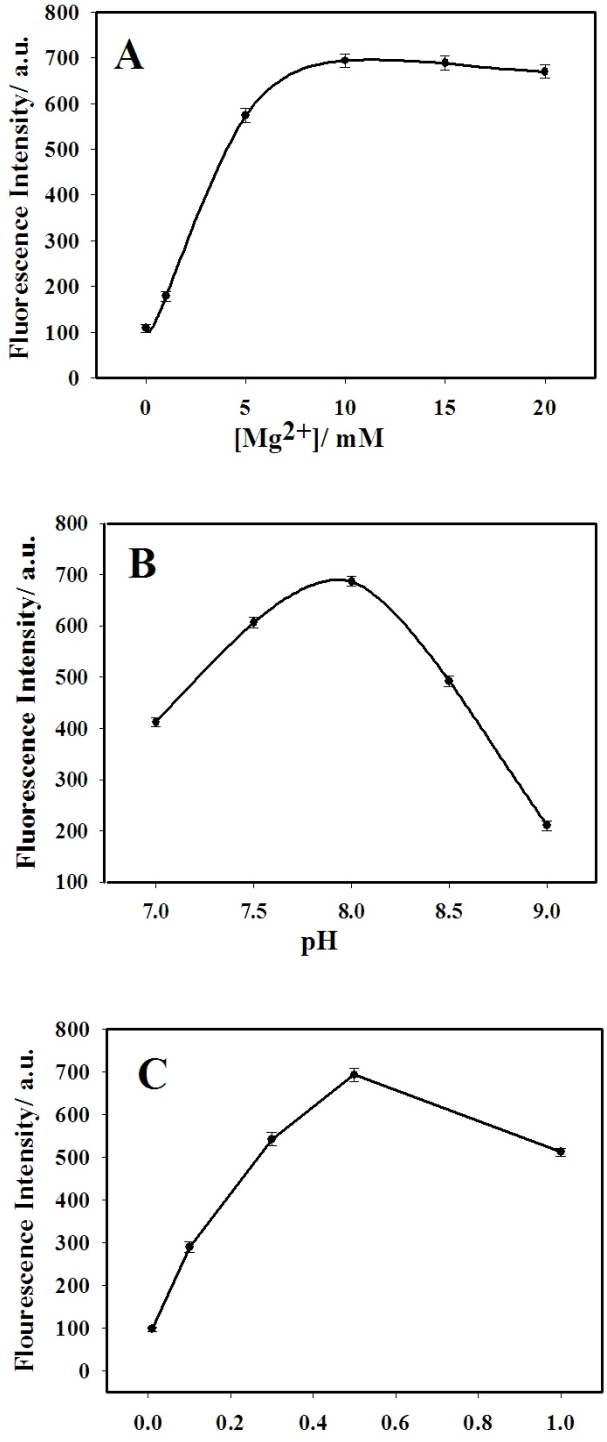


**Fig. S1**. Optimization of $Mg^{2+}$ concentration (A), pH (B) and Exo Ⅲ concentration (C). The concentrations of PNK, ATP and λ Exo were 5 U $mL^{-1}$, 1 mM and 10 units, respectively.

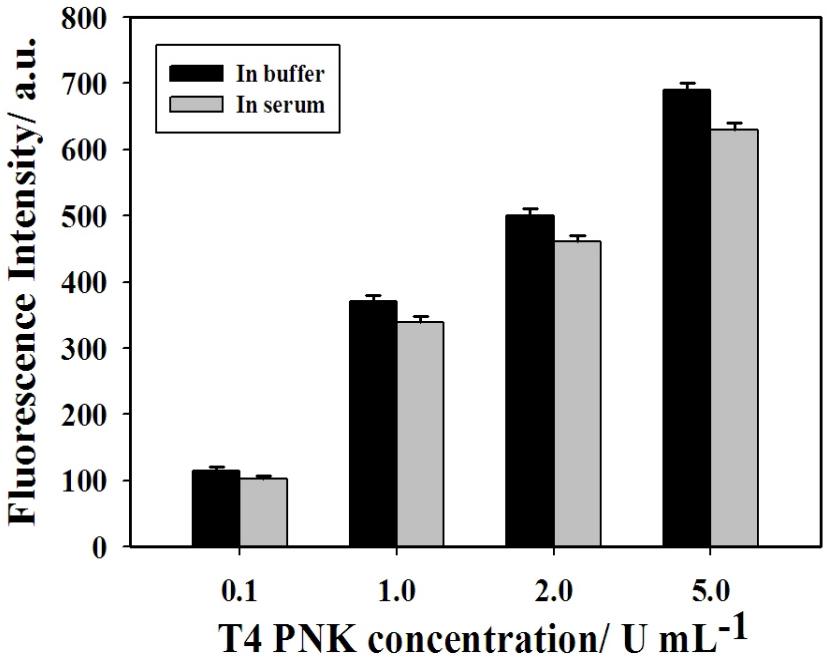


**Fig. S2**. Fluorescence intensity for the fabricated sensing system in buffer and human serum samples spiked with different concentration of T4 PNK. Human serum was diluted in 1:20 with reaction buffer. The error bars represent the standard deviation of three measurements.

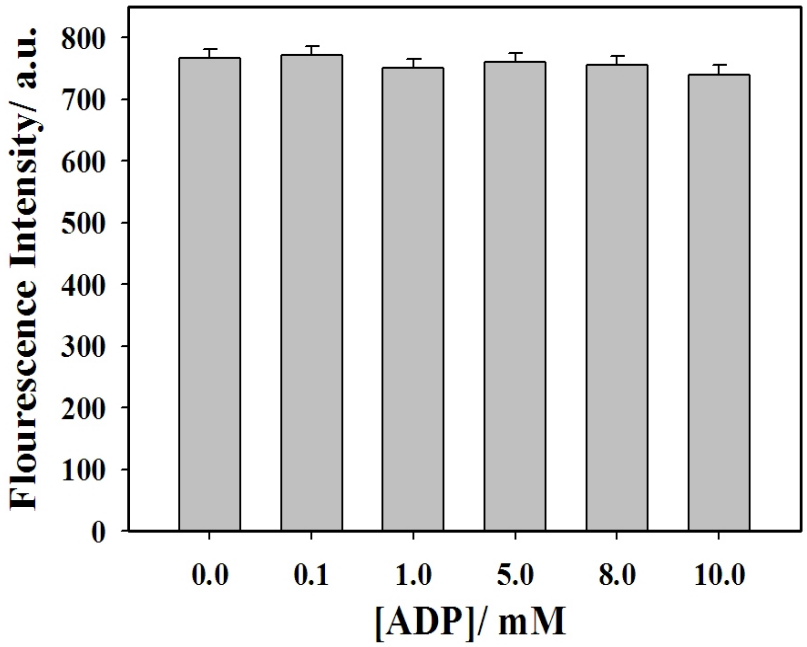


**Fig. S3.** Influence of inhibitor on Exo Ⅲ activity. The assays were carried out with different concentrations of ADP in the reaction buffer.

**Table S2**. Comparison of detection limit for T4 PNK activity based on different methods

| Method | Detection limit | Strategy | Ref. |
|---|---|---|---|
| Fluorescence | 0.04 U $mL^{-1}$ | Smart probe and λ exonuclease cleavage | 1 |
| Fluorescence | 0.05 U $mL^{-1}$ | λ exonuclease reaction and graphene oxide | 2 |
| Fluorescence | 0.49 U $mL^{-1}$ | Copper nanoparticles and λ exonucleas cleavage | 3 |
| Colorimetry | 0.06 U $mL^{-1}$ | HRPzyme and λ exonuclease cleavage | 4 |
| Electrochemistry | 0.01 U $mL^{-1}$ | Titanium ion and SWCNTs | 5 |
| Current measurement | 0.01 U $mL^{-1}$ | Nanochannel and λ exonuclease cleavage | 6 |
| Fluorescence | 0.01 U $mL^{-1}$ | λ exonuclease reaction and Exo Ⅲ amplification | This work |

**References**


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