# Electronic Supplementary Information (ESI)

## A label free fluorescent assay for uracil-DNA glycosylase activity based on the signal amplification of exonuclease I

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## Experimental

### 1. Chemicals and reagents

Uracil-DNA glycosylase, uracil glycosylase inhibitor, M.SssI methyltransfer (M.SssI), Dam methyltransferase (Dam MTase) and Nb.BbvCI restriction endonuclease (Nb.BbvCI) were obtained from New England Biolabs. Exonuclease I was bought from Thermo Fisher Scientific Co. Ltd. (Shanghai, China). 10000×SYBR Green I was purchased from Beijing Solarbio Science & Technology Co., Ltd. 5-Fluorouracil was supplied by Shanghai Sangon Biotechnology Co., Ltd. (China). All other chemicals were of analytical grade and were used as received. The DNA substrates used here were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (China) and the sequences were as follows:

HP: 5'-GCUGUCUGUGAAGGAGGTAGATCACAGACAGC-3'

HP2: 5'-GCTGTCTGTGAAGGAGGTAGATCACAGACAGC-3'

The underlined letters indicate the stem region of DNA probes. U in HP probe is the uracil deoxyribonucleotide modification.

### 2. UDG activity assay

The detection of UDG activity was carried out in 100 μL of enzyme reaction mixture containing 1× reaction buffer (20 mM Tris-HCl, 1.0 mM $MgCl_2$, 1.0 mM EDTA, pH 8.0), 50 nM HP, 1×SG, 20 U Exo I and various concentrations of UDG. The solutions of UDG were diluted with a storage buffer consisting of 50 % glycerol, 20 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.0 mM EDTA, 1.0 mM DTT, 0.10 mg/mL BSA. The mixture was incubated for 20 min at 37℃. And then the fluorescence intensity was

measured using a LS-55 luminescence spectrometer (Perkin-Elmer, USA) at room temperature (25±2℃). The excitation wavelength was 480 nm and the emission spectra were recorded from 505 to 650 nm. The peak intensities at 528 nm were used for the data analysis.

**3. Gel electrophoresis analysis**

In the gel electrophoresis assay, all the contents in the sample were carried out in 20 μL of enzyme reaction mixture containing 1.0 μM HP, 1×SG, 20 U Exo I and 50 U/mL UDG. The electrophoresis analysis was carried out on 4.0 % agarose gel and dissolved in 1×TBE buffer (90 mM Tris, 90 mM boricacid, 10 mM EDTA, pH 8.3). 20 μL different reaction products were added to each lane. The gel was run at constant potential of 100 V for 60 min, and finally photographed with a digital camera under UV light.

**4. UDG activity detection in diluted cell extracts**

NCI-H460 cells $1.0 \times 10^6$ were cultured in RPMI 1640 medium supplemented with 10 % fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin at 37 ℃ under 5.0 % humidified $CO_2$. The harvested cells were washed and then resuspended in 10 mM Tris-HCl. The suspended cells were sonicated with an ultrasonicator for 10 min on ice to obtain crud cell extracts. UDG with various concentrations was prepared with diluted crud cell extracts (2.0 %) and then added into the enzyme assay mixture containing 1.0 μM HP, 1×SG and 20 U Exo I. The detection procedure was the same as those described in the aforementioned experiment for UDG detection in clean reaction buffer.

**5. The UDG inhibition assay**

The inhibition effect of 5-fluorouracil on the activity of UDG was investigated in a solution containing 50 nM HP, 1×SG I, 1.0 U UDG, 20 U Exo I and various concentrations of 5-fluorouracil. The mixtures were incubated at 37 ℃ for 20 min and the fluorescence was then recorded. To investigate the effects of 5-fluorouracil on the digestion ability of Exo I, the solution containing 50 nM HP, 1×SG I, 20 U UDG were incubated at 37℃ for 30 min firstly to obtain a ssDNA-structured probe. Subsequently, 20 U Exo I and 50 mM 5-fluorouracil were added to the solution together. After incubation at 37 ℃ for 20 min, the fluorescence measurement was then implemented.

## Supplementary figures

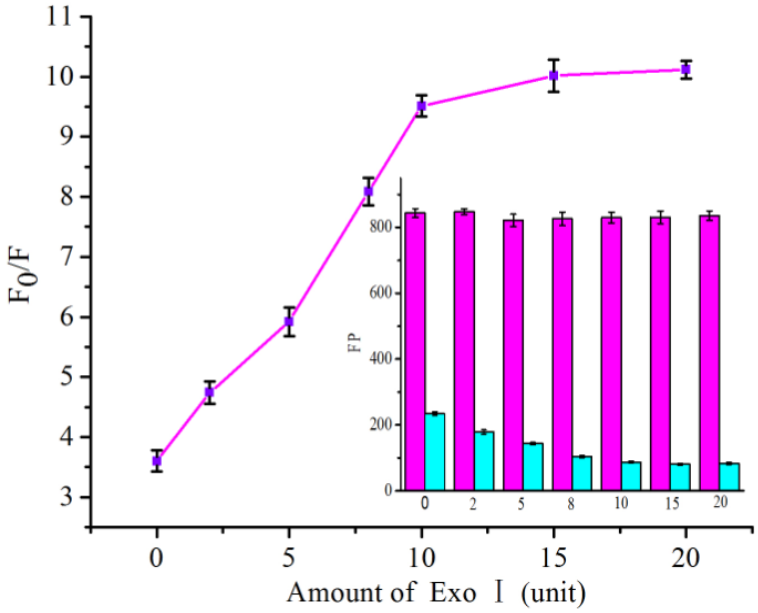


**Fig. S1** The effect of the amount of Exo I on the assay performance. Error bars were estimated from three replicate measurements.

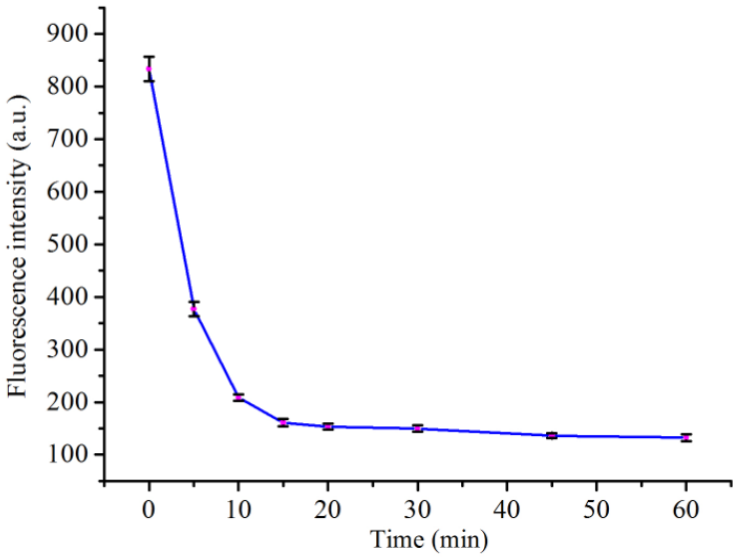


**Fig. S2** The optimization of the reaction time. Error bars were estimated from three replicate measurements.

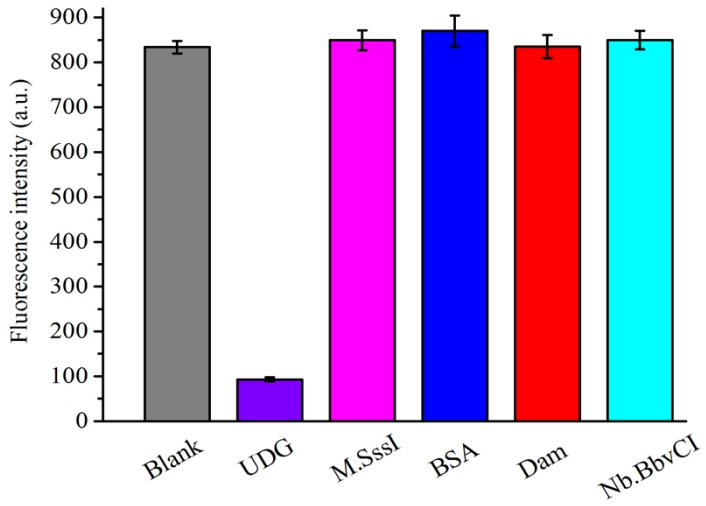


**Fig. S3** Selectivity of the present method. The concentration of UDG was 10 U/mL and it was 40 U/mL for each other control enzyme. The BSA concentration was 200 μg/mL. Error bars were estimated from three replicate measurements.

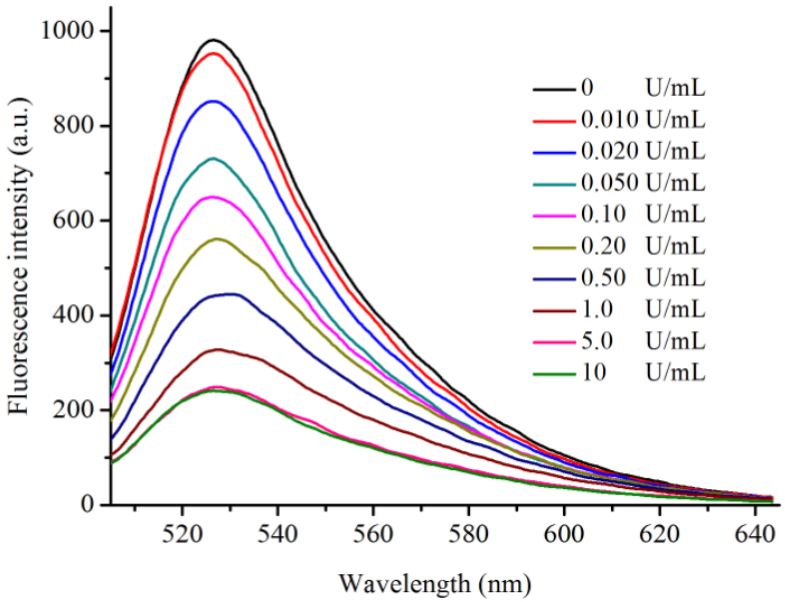


**Fig. S4** Fluorescence spectra in the presence of various concentrations of UDG with diluted cell extract.

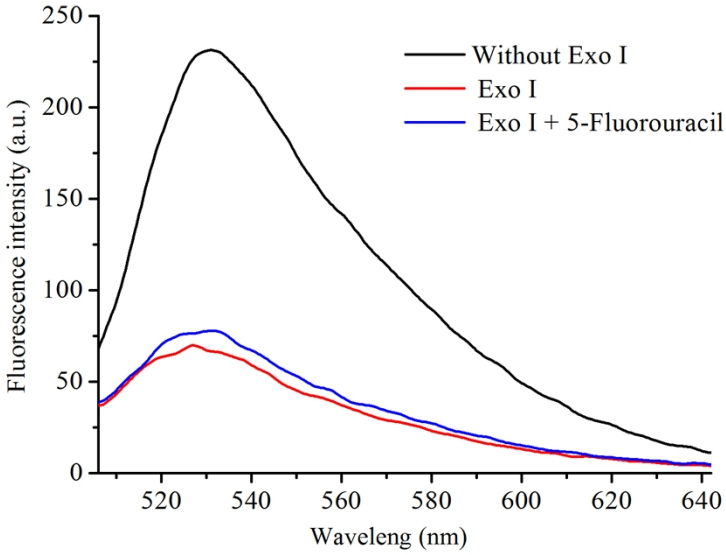


**Fig. S5** Influence of 5-fluorouracil (50 mM) on the activity of Exo I.

# Supplementary table

**Table S1** Comparison of assay performance between the proposed method with other reported methods for UDG activity detection.

| Methods | Total assay time (min) | Linear range (U/mL) | Detection Limit (U/mL) | References |
|---|---|---|---|---|
| Gold nanoparticles colorimetric assay | 30 | 0.5～15 | 0.3 | 1 |
| Nicking enzyme assisted signal amplification-based colorimetric assay | 180 | 0.06～8 | 0.02 | 2 |
| Luminescent G-quadruplex selective switch-on probe | 30 | 0.02～1 | 0.02 | 3 |
| Molecular beacon-based fluorescence sensor | 10 | 0.01～3 | 0.005 | 4 |
| Label free electrochemical biosensor using methylene blue as indicator | >150 min | 0.025～0.1 | 0.012 | 5 |
| DNAzyme amplification strategy for the fluorescent UDG assay | 530 | Not given | 0.002 | 6 |
| Graphene oxide-hairpin probe nanocomposite-based fluorescent assay | 150 | 0.0017-0.2 | 0.0008 | 7 |
| DNAzyme-based fluorescent assay | 11 | 0～0.54 | 0.0051 | 8 |
| Label free fluorescent assay based on the amplification of exonuclease I | 20 | 0.01-5 | 0.007 | This work |

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