Electronic Supplementary Information

Label-Free and High-throughput Bioluminescent Detection of Uracil-DNA Glycosylase in Cancer Cells through Tricyclic Cascade Signal Amplification

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EXPERIMENTAL SECTION

Materials. The oligonucleotides (Table S1) were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). The PolyA and nucleosides triphosphate including deoxythymidine triphosphate（dTTP）and deoxycytidine triphosphate (dCTP) were bought from TaKaRa Bio. Inc. (Dalian, China). Uracil DNA glycosylase (UDG), uracil glycosylase inhibitor (UGI)，terminal transferase (TDT), 10× TDT buffer (500 mM potassium acetate, 200 mM tris-acetate, 100 mM magnesium acetate, pH 7.9), apurinic/apyrimidinic endonuclease 1 (APE1), T7 exonuclease (T7 Exo), 10× NEBuffer 4（500 mM potassium acetate, 200 mM tris-acetate, 100 mM magnesium acetate, 10 mM DTT, pH 7.9), 10 mg/mL bovine serum albumin (BSA)，and human

8-oxoguanine-DNA glycosylase 1 (hOGG1) were obtained from New England BioLabs (Beverly, MA, USA). Phospho(enol)pyruvic acid monosodium salt hydrate (PEP), pyruvate kinase from rabbit muscle (PK), adenylate kinase (AK) from chicken muscle and other chemical reagents were of analytical grade and bought from Sigma-Aldrich, Inc. (St. Louis, MO, USA). The ATP determination kit was purchased from Invitrogen (Carlsbad, CA, USA). The 96-well white microplates were obtained from Fisher Scientific (Pittsburgh, PA, USA). Ultrapure water was prepared by a Millipore filtration system (Millipore, Milford, MA, USA).

Table S1. Sequences of the Synthesized Oligonucleotides

note	sequence (5′-3′)
DNA-1	G*A*T CCT GCA **U**AC TTA GT-ddC
DNA-2	G*A*C TAA GTA TGC AGG AT-ddC
assistant probe	A*A*A AAA AAX AGC AGG AT-ddC
signal probe	$(rA)_{10}(A)_{18}$

[a]The asterisks indicate the phosphorothioate modifications. The bold U base in DNA-1 indicates the damaged uracil deoxyribonucleotide. The ddC indicates 2′,3′-dideoxycytidine. The X in the assistant probe indicates the abasic site mimic. The rA in the signal probe indicates the ribonucleotide.

Preparation of DNA Stock Solutions. The 1 μM DNA-1 probes and 1 μM DNA-2 probes were diluted with 1× annealing buffer containing 50 mM NaCl, 10 mM Tris-HCl (pH 8.0) at 95 °C for 5 min, followed by slowly cooling to room temperature to form the double-strand DNAs (dsDNAs). The obtained dsDNA substrates were stored at −20 °C for further use.

Bioluminescent Detection of UDG. The UDG assay involves three consecutive steps. First, 2 μL of DNA substrates (1 μM) was added into the excision reaction system (10 μL) containing different-concentration UDG, 5 U of APE1 and 1 μL of 10× TDT buffer, followed by incubation at 37 °C for 30 min. Second, 4 U of TDT, 0.5 μL of 10 mM dTTP, 1 μL of 10 μM assistant probe, 2 μL of 2.5 mM $CoCl_2$, and 1 μL of 10× TDT buffer were added into the excision reaction system with a total volume of 20 μL, followed by incubation at 37 °C for 60 min for amplification reaction. The reaction was terminated by inactivation at 95 °C for 10 min. Third, 1.4 μL of signal probe (10 μM), 2 U of T7 Exo, 5 μL of 10× NEBuffer 4 were added into the amplification reaction system with a total volume of 50 μL, and then incubated at 37 °C for 30 min for hydrolysis reaction. Finally, all the reaction products were transferred into the ATP detection system for bioluminescent assay in a total volume of 60 μL. The ATP detection system consisted of 4.0 μL of AMP-to-ATP conversion buffer (1.0 μL of 1 U/μL AK, 1.0 μL of 1 U/μL PK, 1.0 μL of 10 mM dCTP, and 1.0 μL of 4.8 mM PEP), and 6.0 μL of ATP detection buffer (0.5 mM D -luciferin, 1.25 μg/mL firefly luciferase, 10 mg/mL BSA, 500 mM Tricine buffer (pH 7.8), 100 mM $MgSO_4$, 2 mM EDTA, and 100 mM DTT). After all the above reagents were mixed, the bioluminescence signal was monitored by a Glomax luminometer (Promega, Madison, WI, USA) at room temperature.

Gel Electrophoresis. To analyze the amplification reaction products, the 12% nondenaturating polyacry-lamide gel electrophoresis (PAGE) was performed in 1× TBE buffer (9 mM boric acid, 9 mM Tris-HCl, 0.2 mM EDTA, pH 7.9) using 1× SYBR Gold as the indicator at 110 V constant voltage for 45 min at room temperature.

Inhibition Assay. Different-concentration UGI was incubated with the DNA substrates at 25 °C for 15 min, and then 5 U/mL UDG was added into the solution and incubated at 37 °C for 60 min. The following reactions and bioluminescence measurement were performed with the procedures described above. The relative activity (RA) of UDG was measured according to RA (%) = $\frac{B_i - B_0}{B_t - B_0} \times 100\%$, where B_0 is the bioluminescence intensity in the absence of UDG, B_t is the bioluminescence intensity in the presence of 5 U/mL UDG, and B_i is the bioluminescence intensity in the presence of UDG and UGI.

Cell Culture and Preparation of Cell Extracts. Human cervical carcinoma cell line (HeLa cells) was cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS, Invitrogen, USA), 1% penicillin-streptomycin (Invitrogen, USA), and cultured under a 5% CO_2 atmosphere at 37°C. In the exponential phase of growth, the number of cells was measured by Countstar cell counter. The nuclear extracts were prepared using the nuclear extract kit (ActiveMotif, Carlsbad, CA, USA) according to the manufacturer's protocol.

SUPPLEMENTARY RESULTS

Optimization of Experimental Conditions. To obtain the best performance, we investigated the effect of APE1 amount, T7 Exo amount, T7 Exo reaction temperature and concentration of signal probe upon the bioluminescence signal (Figure S1). As shown in Figure S1A, the B/B_0 value improves with the increasing amount of APE1 from 1 to 5 U, followed by the decrease beyond the amount of 5 U (B and B_0 are the bioluminescence intensity in the presence and absence of UDG,

respectively). Thus, 5 U of APE1 is used in the subsequent research. The influence of T7 Exo upon the bioluminescence signal was also investigated with a fixed amount of APE1 (5 U). As shown in Figure S1B, the B/B_0 value improves with the increasing T7 Exo amount and reaches a plateau at the amount of 2 U (B and B_0 are the bioluminescence intensity in the presence and absence of UDG, respectively). Thus, 2 U of T7 Exo is used in the subsequent research. We further optimized the T7 Exo reaction temperature and the concentration of signal probe. As shown in Figure S1C, the B/B_0 value in response to 37°C is much higher than that in response to 25°C and 45°C (B and B_0 are the bioluminescence intensity in the presence and absence of UDG, respectively). Thus, the reaction temperature is set at 37°C in the subsequent experiments. Only the signal probe strand in DNA-RNA hybrids can be degraded by T7 Exo to release AMPs for the production of bioluminescence signal, and thus the concentration of signal probe has a crucial effect on the bioluminescence signal. The high-concentration signal probes will result in the high hybridization efficiency and consequently the high digestion efficiency and the high bioluminescence signal, but the high-concentration signal probes might cause the dimerization of signal probes and increase the background signal. As shown in Figure S1D, the B/B_0 value improves with the increasing concentration of signal probe from 0.12 to 0.28 μM and reaches the highest value at 0.28 μM (where B and B_0 are the bioluminescence intensity in the presence and absence of UDG, respectively). Thus, 0.28 μM signal probe is used in the subsequent research.

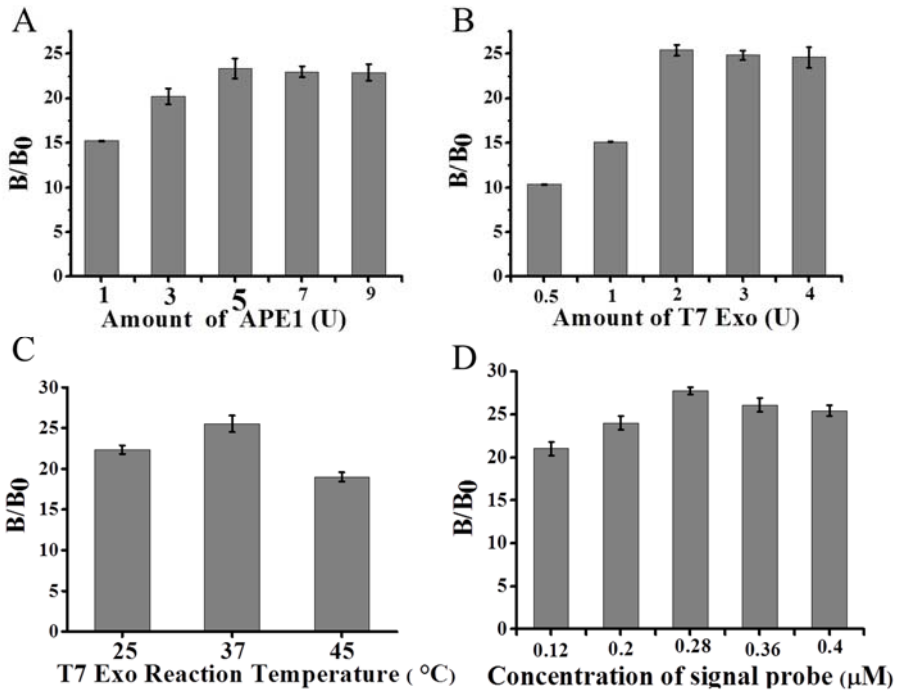

Figure S1. (A)Variance of B/B_0 value with the amount of APE1 at a fixed amount of T7 Exo (3 U); (B) Variance of B/B_0 value with the amount of T7 Exo at a fixed amount of APE1 (5 U); (C) Variance of B/B_0 value with the T7 Exo reaction temperature; (D) Variance of B/B_0 value with the concentration of signal probe. B and B_0 are the bioluminescence intensity in the presence and absence of UDG, respectively. The UDG concentration is 50 U/mL. Error bars represent the standard deviation of three independent experiments.

Kinetic Analysis.

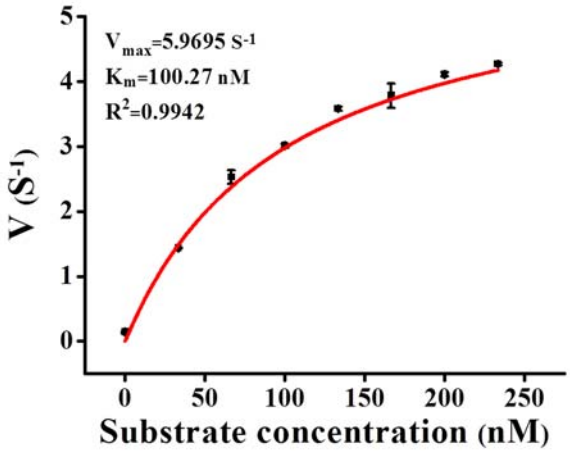

Figure S2. Variance of initial velocity with the concentration of DNA substrate in response to 50 U/mL UDG. Error bars show the standard deviation of three experiments.