Electronic Supporting Information

A Sensitive, Homogeneous Fluorescence Assay of Thymine DNA Glycosylase Activity with Exonuclease-Mediated Amplification

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Experiment Section

Reagents.

Oligonucleotides (DNA probe, control DNA and Taqman reporter) used in this work were customer designed and synthesized without any base modification and purified by ultrapage by Sangon Biotechnology Co. Ltd. (Shanghai, China), as well as 5× TBE (225 mM Tris- boric Acid, 50mM EDTA, pH 8.0). The sequences of the synthesized oligonucleotides were given in Table S1. High concentration DNA stock solutions was prepared by steriled water. The prepared probes were kept at 4 °C before use to minimize denaturation. SYBR Green I (10,000× concentrate) was supplied by Life Technologies corporation (USA). Ultra Low Range DNA Ladder supplied with 6× DNA Loading Dye (10 mM Tris-HCl, 0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% propanetriol, 60 mM EDTA, pH 7.6) was obtained from Thermo Fisher Scientific Inc. (USA). Endonuclease IV, T7 Exonuclease, 8-Oxoguanine DNA Glycosylase (hOGG1), Bovine Serum Albumin (BSA) and 10× NEBuffer 4 (200 mM Tris-acetate (pH 7.9), 500 mM Potassium Acetate, 100 mM Magnesium Acetate, 10 mM Dithiothreitol) were purchased from New England Biolabs Ltd. (USA). Thymine DNA Glycosylase (TDG) was bought from R&D System (USA). Prostate specific antigen (PSA) was obtained from Invitrogen Corporation. (USA). GoldView, ethidium bromide and rabbit anti-human IgG were provided by Beijing Dingguo Changsheng Biotechnology Co. Ltd. (China).

Apparatus and measurements.

Ultrapure water used to prepare all of the solutions was obtained through a Nanopure Infinity Ultrapure Water System (Barnstead/Thermolyne Corp., Dubuque, IA) with an electrical resistance larger than 18.3 MΩ. All buffer solutions and ultrapure water were sterilized and used throughout experiments. Fluorescence experiments were all performed using a Hitachi F-7000 fluorescence spectrometer (Hitachi. Ltd., Japan) controlled by FL Solution software for curve-fitting and peak height determination at room temperature. A quartz fluorescence cell with an optical path length of 1.0 cm was used. The excitation

was made at 494 nm with a recording emission range from 510 to 600 nm. All excitation and emission slits were set at 5 nm with the voltage of 950 V.

Thermodynamic parameters and secondary structures of all oligonucleotides were calculated using bioinformatics software (http://www.bioin-fo.rpi.edu/applications/).

Fluorescence Measurements of TDG Activity.

The typical TDG activity assay was performed at 65 ºC for 2 h in a 30μL 1 × NEbuffer 4 containing 50 nM DNA hairpin probe and varying concentration of TDG (ranging from 0 to 0.17 U μL^{-1}). Then, EnIV (0.33 U μL^{-1}), T7 Exo (1 U μL^{-1}) and 200 nM Taqman reporter were added to the mixture and incubated at 25 ºC for 2 h. The resulting solution was subjected to fluorescence measurements.

Note: For practical applications excessive duplex DNA in the sample will not affect the T7 Exo activity, because we use excessive T7 exo in our assay. If a great amount of DNA is present, more T7 exo (3 U μL^{-1}) may be required to eliminate the effect.

Melting Curve Analysis.

To verify TDG enzyme can recognize T/G mismatch in duplex DNA and cleave the strand with the T in probe. Samples were prepared by 3 μM DNA probe in 20 μL of 1×NEBuffer 4 solutions in the absence or presence of TDG heating at 65 ºC for 2h, followed by the second step at 25 ºC as well by adding EnIV (0.33 U μL^{-1}) and then T7 Exo (1 U μL^{-1}) in 30 μL of 1× NEBuffer 4 solutions. For gel electrophoresis analysis, the reaction conditions were the same as those for the given DNA samples.

6 μL of the former reaction solution was taken and was added into a total of 25 μL 1× NEBuffer 4 mixture containing 1×conc. SYBR Green I. The melting curve of the mixture was recorded using a C1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA) with a CFX 96 in situ detection system. The detecting solution was heated from 45 ºC to 95 ºC with an increment rate of 1.0 ºC /5 s. The fluorescent signals were obtained throughout the melting analysis process, and the data was analyzed using CFX Manager Software.

Gel Electrophoresis Analysis of Thymine DNA Glycosylase Activity.

The resultant mixture used was the same as the reaction solution before melting curve analysis and was analyzed using gel electrophoresis in 3% (w/w) agarose containing 0.5 μg mL^{-1} GoldView and the same concentration of ethidium bromide running in 0.5× TBE (22.5 mM Tris-Boric acid, 5 mM EDTA, pH 8.0) at room temperature. Electrophoresis was performed at a constant potential of 140 V for 90 min with loading of 9 μL of each sample into the lanes. The gel was visualized using a Tocan 240 gel imaging system (Shanghai Tocan Biotechnology Company).

Cell culure and sample preparation.

HeLa, MCF-7 and MCF-10A cells were cultured in RPMI 1640 medium (Thermo Scientific HyClone) supplemented with 15% heat-inactivated fetal bovine serum and 0.1 U μL^{-1} penicillin and 0.1 g μL^{-1} streptomycin. These cells were maintained at 37 ºC in a humidified atmosphere containing 5% CO_2.

The whole-cell extracts were prepared as follows: Cells (5×10^5, 1×10^6 or 2×10^6 cells) were dispensed in a 1.5 mL centrifuge tube, washed twice with phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM Phosphate Buffer, pH 7.4) at 2000 rpm for 3 min, and then suspended in 100 μL of lysis buffer (10 mM Tris-HCl with pH 8.0, 150 mM NaCl, 1%(w/v) NP-40, 0.25 mM sodium deoxycholate, 1%(w/v) glycerol and 0.1 mM 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride). The lysates were incubated for 30 min on ice and vortex for 30 s every 5 min. Then the cell debris was removed by centrifugation at 12,000 rpm for 20 min at 4 ºC. The supernate was used immediately for TDG activity assay.

As for the cytoplasm extracts, the operation steps were fully in accordance with the instruction manual of the cytoplasmic extraction kit from Beyotime Institute of Biotechnology (Nantong, China) with the cytoplasmic protein containing 1×10^6 cells μL^{-1} before disintegrated.

In a 20 μL aliquot of reagent solution containing 75 nM DNA hairpin probe in 1× NEbuffer 4, 1 μL lysis buffer, whole-cell extracts and cytoplasm extract with different cell numbers was added. The mixtures were incubating at 37 ºC for 2 h, followed by mixed into EnIV (0.33 U μL^{-1}) and T7 Exo (1 U μL^{-1}) in a total of 30 μL of 1×NEBuffer 4 solutions heating at 25 ºC for 2 h, too. These samples were immediately detected fluorescence.

Table S1 Sequences of the synthesized oligonucleotides

Probes	Sequence (5’ to 3’)
DNA Probe	TAT ATA TGG TAG TGA GTA GTG AGG TAG GTT GTA TAG TTG AGG TAA ATT ATA CAA CCT ACC
Control Probe	TAT ATA TGG TAG TGA GTA GTG AGG TAG GTT GTA TAG TTG AGG TAA ACT ATA CAA CCT ACC
Taqman Reporter	FAM-**A**AC (TAMRA)**T**AT ACA AC

Table S2 Comparsion of TDG detection sensitivity with different methods

Method	Detecion method	Detection range	Reference
DNA cleavage followed by gel electrophresis	Fluormetric or Radioactive image	N/A	1, 2
ELISA based 5-fc detecion by TDG excision	Colormetric	50 -1000 ng /mL with a detection limit of 20 ng / mL	3
Sandwitch ELISA	Colormetric	0.312 -20 ng / mL with a detection limit of 0.038 ng / mL	4
DNA probe with exonuclease-mediated amplification	Fluormetric	0.046 -43.4 ng / mL with a detection limit 0.046 ng / mL	This work

Reference

1. Y. Q. Li, P. Z. Zhou, X. D. Zheng, C. P. Walshand and G. L. Xu, *Nucl. Acids. Res.*, 2007, **35**, 390. (same as Ref 11 in manuscipt)
2. U. Hardeland, M. Bentele, J. Jiricny, and Primo Schar, *J Biol Chem*, 2000, 275, 33449. (same as Ref 12 in manuscript)
3. Method from Epigentek Group Inc. (on the market since 2012) http://www.epigentek.com/catalog/epigenase-thymine-dna-glycosylase-tdg-activityinhibition-assay-kit-colorimetric-p-3017.html (accessed date 04/26/13).
4.Method from antibodies-online Inc. http://www.antibodies-online.com/kit/422664/Thymine-DNA+Glycosylase+TDG+ELISA/(accessed date 04/26/13)

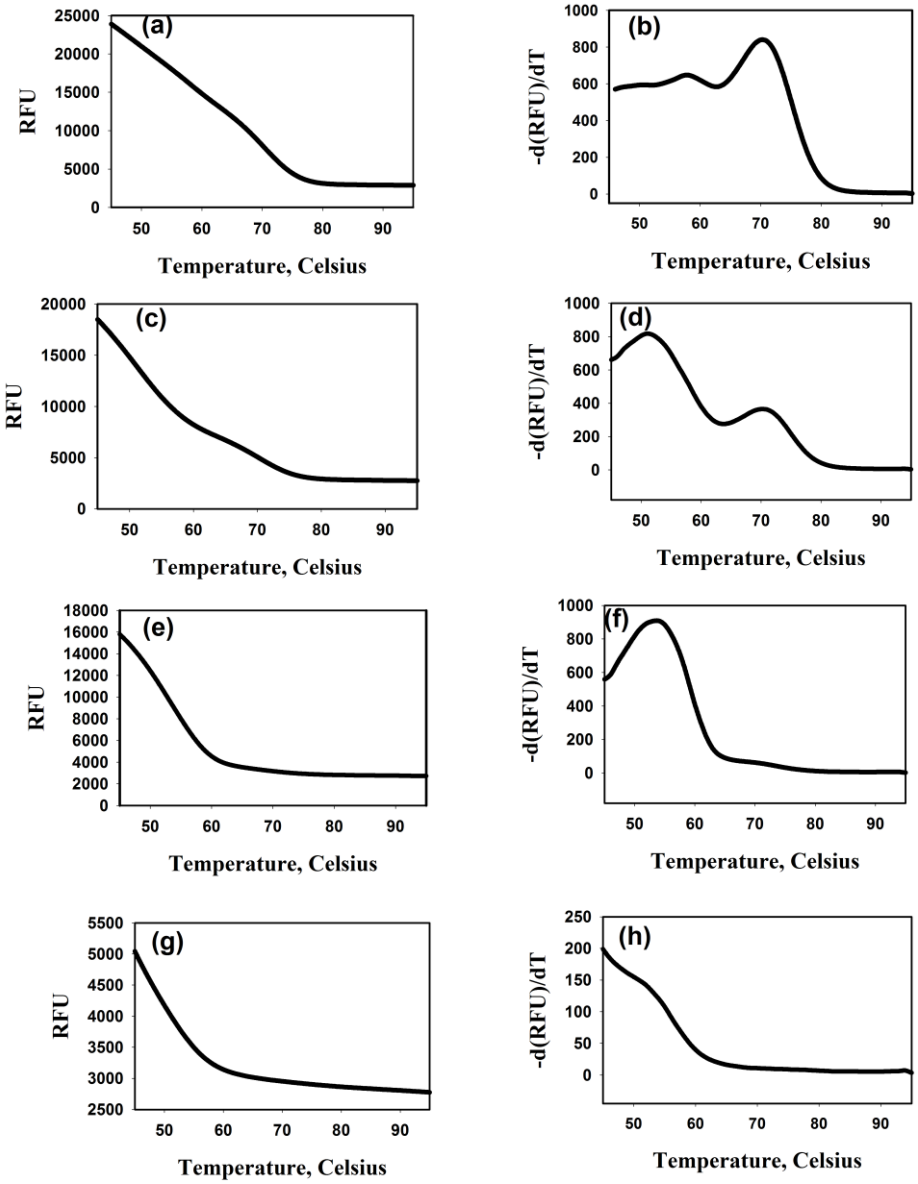

Fig. S1 Melting analysis. In the left column are fluorescence intensity curves (a, c, e, g) and in the right column are corresponding derivative curve (b, d, f, h). a and b, only DNA probe; c and d, DNA probe plus TDG; e and f, DNA probe plus TDG and EnIV, g and h, DNA probe plus TDG, EnIV and T7 Exo.

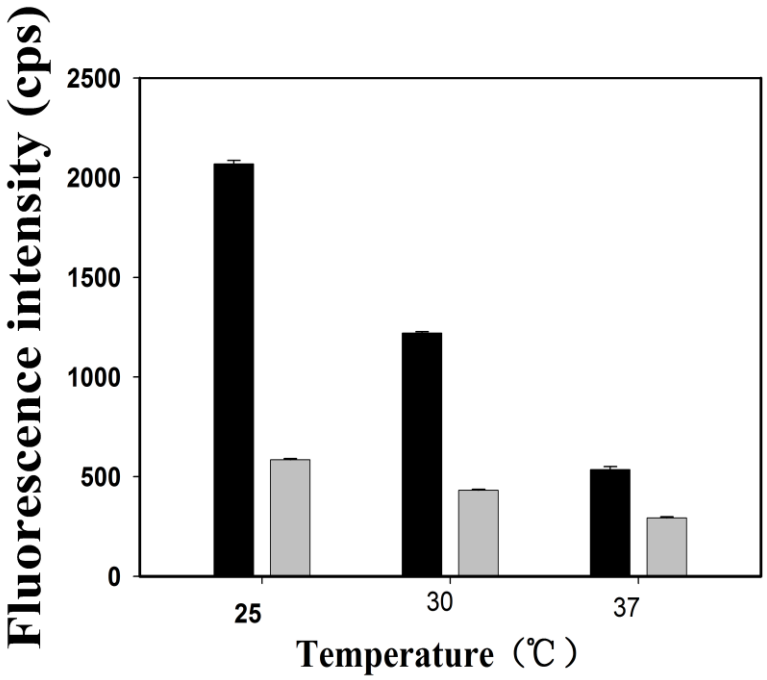

Fig. S2 Bar plot of corresponding fluorescence responses at 520 nm of TDG activity assay in 1× NEBuffer 4: containing 50 nM DNA probe, TDG (0.013 U $\mu L^{-1)}$, T7 Exo (1 U μL^{-1}) and TaqMan reporter (200 nM) in the presence of EnIV (0.33 U μL^{-1}) (black) or in the absence of EnIV (grey) at different temperature. Error bars were estimated from at least three independent measurements.

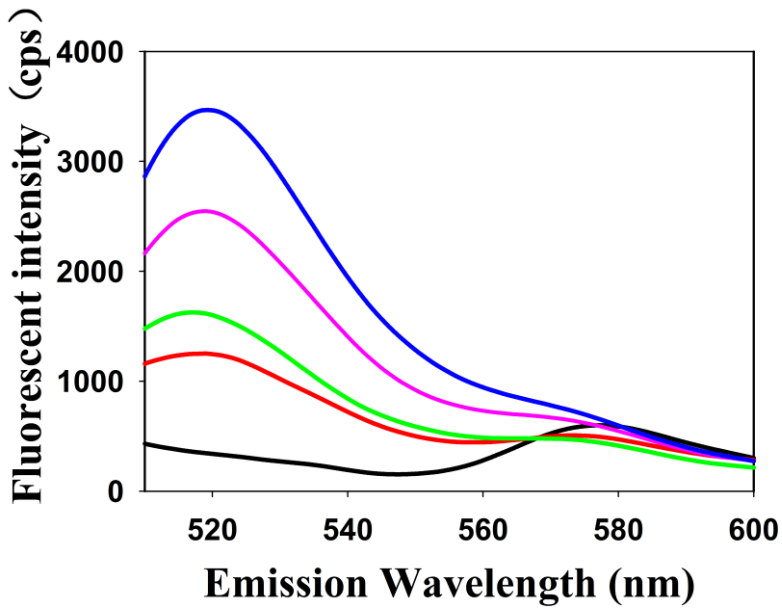

Fig. S3 Fluorescence spectra obtained from cell extracts with different Hela cell concentrations. Lysis buffer(black), 333.3 cells μL^{-1} cytoplasm extracts (red), 166.6 cells μL^{-1} whole-cell extract (green), 333.3 cells μL^{-1} whole cell extract (pink) and 666.6 cells μL^{-1} whole-cell extract (blue). The results were the average of three experiments.

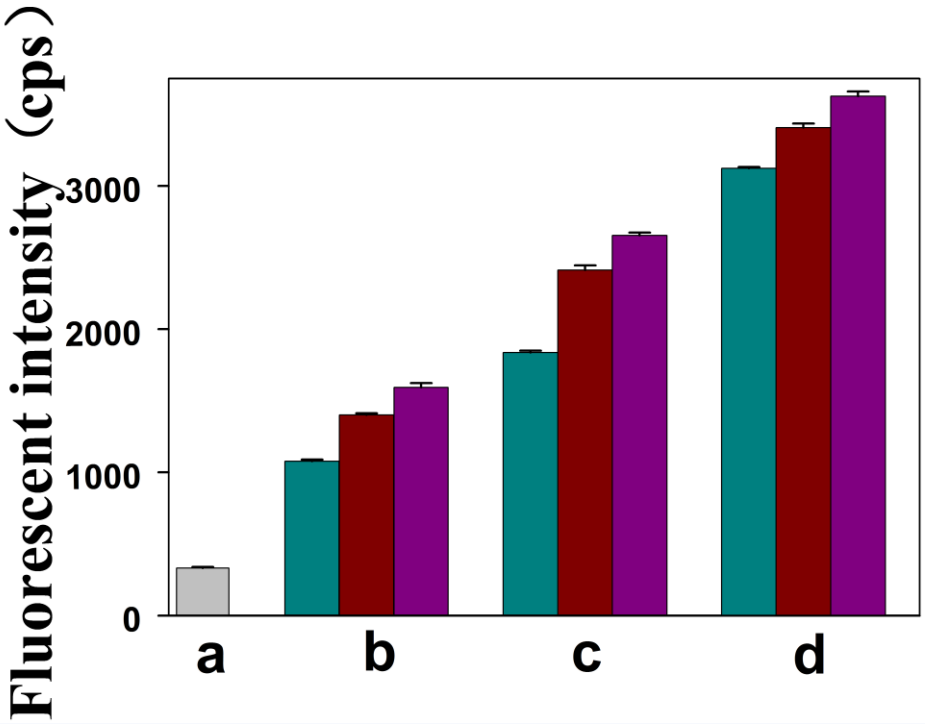

Fig. S4 Fluorescence spectra obtained from cell extracts of different kind of cells. (a) only whole cell lysis buffer. b, c, and d represent 166.6, 333.3, 666.6 cells μL^{-1} cell extract from whole cell. Dark cyan, dark red and dark pink was used for standing for cell extracts from Human breast cancer MCF-7 cells, Human breast epithelial MCF-10A cells and Human cervical cancer Hela cells.