Electronic Supplementary Information

A simple "mix-and-detection" method for sensitive detection of telomerase from cancer cells under absolutely isothermal condition

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EXPERIMENTAL SECTION

Chemicals and Materials. All oligonucleotides (Table S1) were synthesized by TaKaRa Bio. Inc. (Dalian, China). The Phi29 DNA polymerase, NB.BbvCI nicking endonuclease, and four mixed nucleotides (dNTPs, including dATPs, dTTPs, dCTPs, and dGTPs) were purchased from New England Biolabs (Beverly, MA). SYBR Green Gold was obtained from Invitrogen (Carlsbad, CA, USA). TRAPeze 1× CHAPS lysis buffer was purchased from Millipore (Bedford, MA, USA). All other chemical agents were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water

obtained from a Millipore filtration system was used in all experiments.

Table S1. Sequence of oligonucleotides [a]

note	sequence (5'-3')
telomeric primer	CCC TTA CCC TTA CCC TTA CCC TAA
detection probe	AAC TAT ACA ACC TAC TAC CTC ACC TCA GC*T ACA ATC CGT CGA GCA GAG TT*
circular template	ATC TAT AGA CCT CAG CTC GTA CTA GCA CAA ACT ATA CAA CCT ACT ACC TCA GAT GAG CTA

[a]The underlined bases indicate the recognition sequences of Nb.BbvCI nicking endonuclease. The italic bases indicate the telomerase substrate sequences.

Cell Culture and Preparation of Telomerase Extracts. Different cell lines including normal lung cell line (MRC-5 cells), cervical cancer cell line (Hela cells), lung cancer cell line (A549 cells), and embryonic kidney cell line (HEK293T cells) were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum and 50 U mL^{-1} penicillin plus 50 μg mL^{-1} streptomycin in a humidified chamber containing 5% CO_2 at 37 °C. For telomerase extraction, cells in the exponential phase of growth were collected and resuspended in 200 μL of ice-cold 1× CHAPS lysis buffer (0.5% CHAPS, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 5 mM mercaptoethanol, 10% glycerol, 10 mM Tris-HCl, pH 7.5), and incubated on ice for 30 min, followed by centrifugation at 12000 g for 20 min at 4 °C. After centrifugation, the supernatant was carefully transferred into a fresh tube and stored at -80 °C until use.

One-Step Telomerase Detection. The whole reaction was performed in 50 μL of solution containing 2 μL of telomerase extracts, 20 nM probes, 20 nM telomeric primer, 10 nM circular template, 1× SYBR Gold, 20 mM Tris-HCl (pH 8.5), 5 mM MgCl, 10 mM $(NH4)_2SO_4$, 20 mM KCl, 1 mM EGTA, 0.05% (v/v) Tween 20, 0.25 mM dNTPs, 1 U of Phi29 DNA polymerase, and 0.2 U of Nb.BbvC. After reaction at 35 °C for 2 h, the fluorescence spectra were measured by a Hitachi F-4500 fluorometer (Tokyo, Japan) with an excitation wavelength of 495 nm, and the spectra were recorded between 500 and 700 nm. The fluorescence intensity at 540 nm was used for data analysis.

Gel Electrophoresis. The amplification products were analyzed by 2% agarose gel electrophoresis in 1× TAE buffer at a 120 V constant voltage for 40 min. The gels were stained by SYBR gold and analyzed by ChemiDoc MP Imaging System (Bio-Rad, Hercules, CA, USA).

Inhibition Assay. The Hela cells were cultured in serum-containing Dulbecco's modified Eagle's medium and incubated for 6 h prior to the addition of inhibitor MST-312 [N,N'-bis (2,3-dihydroxybenzoyl)-1,2-phenylenediamine]. After incubation with various-concentration MST-312 for 72 h, the cells were collected for telomerase extraction according to the procedures mentioned above, and the telomerase activity of 1000 cells was measured by the proposed method. The relative enzyme activity is quantitatively measured according to eq. 1.

$$\text{Relative activity}\ (\%) = \frac{F_{\mathrm{i}} - F_0}{F - F_0} \times 100\% \qquad (1)$$

where F_0, F and F_{i} represent the fluorescence intensity in the absence of telomerase extracts, in the presence of telomerase extracts, and in the presence of both telomerase extracts and MST-312, respectively.

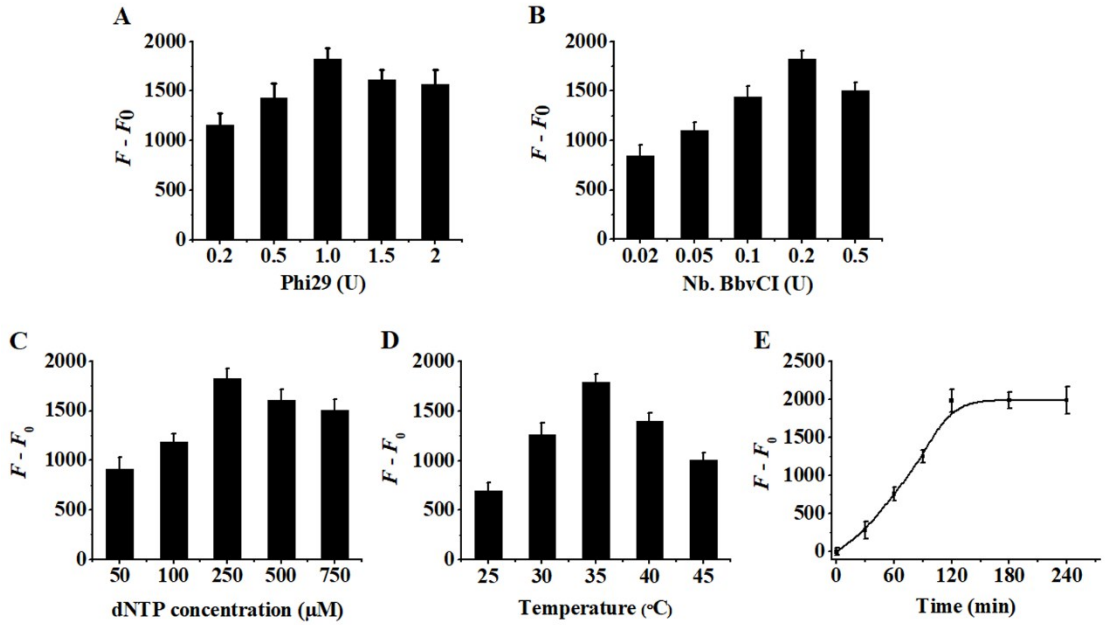

Fig. S1 (A) Variance of the $F - F_0$ value with the amount of Phi29 DNA polymerase. (B) Variance of the $F - F_0$ value with the amount of Nb.BbvCI nicking endonuclease. (C) Variance of the $F - F_0$ value with the concentration of dNTP. (D) Variance of the $F - F_0$ value with the reaction temperature. (E) Variance of the $F - F_0$ value with the reaction time. F and F_0 represent the fluorescence signals in the presence and absence of telomerase extracts. The telomerase extracts are equivalent to 10^5 Hela cells. The error bars represent the standard deviations of three experiments.

Optimization of Experimental Conditions. To obtain the best assay performance, we optimized different experimental conditions including the reaction temperature, the concentration of dNTP, the amount of Nb.BbvCI nicking endonuclease, the amount of Phi29 DNA polymerase, and the reaction time (Fig. S1). We employed the fluorescence value of $F - F_0$ for quantitative analysis, where F and F_0 represent the fluorescence intensity in the presence and absence of telomerase extracts. In this assay, the signal amplification is achieved by the cooperation between Phi29 DNA

polymerase and Nb.BbvCI nicking endonuclease, and thus the amounts of Phi29 DNA polymerase and Nb.BbvCI nicking endonuclease should be optimized (Fig. S1A). When the amount of Nb.BbvCI nicking endonuclease is fixed at 0.2 U, the value of $F - F_0$ enhances with the increasing amount of Phi29 DNA polymerase from 0.2 to 1 U, and levels off beyond the amount of 1 U. Therefore, 1 U of Phi29 DNA polymerase is used in the subsequent researches. When the amount of Phi29 DNA polymerase is fixed at 1 U, the value of $F - F_0$ enhances with the increasing amount of Nb.BbvCI nicking endonuclease from 0.02 to 0.2 U, followed by the decrease beyond the amount of 0.2 U (Fig. S1B). Therefore, 0.2 U of Nb.BbvCI nicking endonuclease is used in the subsequent experiments.

The dNTP is the fuel of amplification system and may influence the amplification efficiencies of both target and background.[1] As shown in Fig. S1C, the value of $F - F_0$ improves with the increasing concentration of dNTP from 50 to 250 μM, followed by the decrease beyond the concentration of 250 μM. Therefore, 250 μM is selected as the optimized dNTP concentration.

The temperature greatly affects the activities of telomerase, Phi29 DNA polymerase, and Nb.BbvCI nicking endonuclease, which will in turn affect the amplification efficiency. As shown in Fig. S1D, the value of $F - F_0$ improves with the increase of reaction temperature from 25 to 35 °C, followed by the decrease beyond 35 °C. Therefore, the reaction temperature of 35 °C is used in the subsequent experiments.

We further investigated the effect of reaction time upon assay performance. As shown in Fig. S1E, the $F - F_0$ value increases rapidly with the reaction time and reaches a plateau within 120 min, indicating the completion of telomerase-triggered isothermal amplification within 120 min. Therefore, the reaction time of 120 min is used in the subsequent researches.

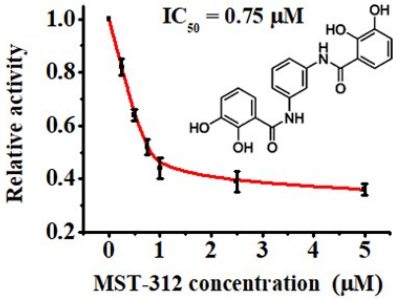

Fig. S2 Inhibition of telomerase activity in Hela cells by different-concentration MST-312 ranging from 0 to 5 μM. Inset shows the chemical structure of MST-312. The error bars represent the standard deviations of three experiments.

Table S2. Comparison of the proposed method with the reported methods for telomerase assay [a]

strategy	requirement of labeled probes	assay time*	assay temperature (°C)	LOD	Ref.
fluorescent assay based on PG-RCA	no	~ 2 h	35	3 Hela cells	this assay
fluorescent assay based on EXPIATR	yes (Iowa Black and FAM)	~25 min	37 and 55	1 Hela cell	2
fluorescent assay based on single QD	yes (biotin and Cy5)	~ 1.5 h	37, 95 and room temperature	185 Hela cells	3
fluorescent assay based on T7 exonuclease-assisted target recycling amplification	Yes (FAM and TAMRA)	~ 3 h	30 and 37	5 Hela cells	4
fluorescent assay based on primer-functionalized QDs	yes (thiol)	~ 4h	38	270 HEK293T cells/μL	5
electrochemical assay based on gold nanoparticles triggered mimic-HCR	no	~ 21 h	37 and room temperature	2 Hela cells	6

ECL assay based on luminol-modified AuNPs	no	over 24 h	37	313 HL-60 cells	7
ECL assay based on magnetic separation	yes (biotin and thiol)	over 24 h	30, 37 and 40	500 Hela cells	8
colorimetric assay based on primer-modified AuNPs	yes (thiol)	over 24 h	30 and room temperature	5 Hela cells	9
chemiluminescence assay based on G-quadruplex-hemin DNAzyme	yes (thiol)	over 3 days	37 and 30	100 Hela cells	10

[a]QD: quantum dot; EXPIATR: exponential isothermal amplification of telomere repeat; HCR: hybridization chain reaction; ECL: electrochemiluminescence; AuNP: gold nanoparticle; LOD: limit of detection.

*Assay time includes the preparation time.

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