Supporting Information for

An ultrasensitive chemiluminescence strategy based on the microchip platform for telomerase detection at single-cell level

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1. Experimental Section

1.1 Reagents and materials

The T7 exonuclease, dNTPs and 10×NEBuffer 4 (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, pH 7.9) were purchased from New England Biolabs Co., Ltd. (Beijing, China). Hydrogen peroxide, sodium hydroxide, sodium dodecyl sulfate (SDS), borax and hydrochloric acid were purchased from Sinoponics Group Chemical Reagent Co., Ltd. (Shanghai, China). Luminol was purchased from Sigma-Aldrich (Saint Louis, MO, USA). DNA Marker, 5×TBE, diethyl pyrocarbonate treated water (DEPC water) were purchased from Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). DMEM hyclone and RPMI1640 hyclone were purchased from Thermo Fisher Scientific Inc. Fetal bovine serum was purchased from Shanghai ExCell Biology Inc. Trypsin-EDTA digestive juice (0.25%), TE buffer（pH8.0）and penicillin-streptomycin solution were purchased from Beijing Solarbio Science & Technology Co., Ltd. NP-40 lysate was purchased from Biyun Biotechnology Co., Ltd. The DNA sequences were synthesized by Takara Biomedical Technology (Dalian) Co., Ltd, the sequence was as shown in Table S1. Hela, A549 and HL-7702 cell lines were obtained from the cell/stem cell bank of the Chinese Academy of Sciences. Electrophoresis buffer was 25 mM borate solution (pH 9.5) containing 20 mM SDS. Chemiluminescent oxidant solution was 35 mM $NaHCO_3$ buffer (pH10.5) containing 1.0 mM PIP and 130 mM H_2O_2. All solutions were filtered through a 0.45 μm membrane filter.

1.2 Apparatus and microfluidic chip

The MCE-CL system was designed and constructed by our laboratory. The structure of the instrument was the same as the previous reported.[1] It consisted of an intelligent 8-channel high voltage power supply (0~5000 V), a microfluidic glass chip, a the photomultiplier tube (PMT) and a data acquisition system (chromatography workstation HW-2000). The microchip was purchased from Dalian Tuo Microchip Technology Co., Ltd. The chip design was as shown in Fig. S1.

1.3 Cells culture

A549 cells and HL-7702 cells were grown in DMEM medium containing 10% fetal bovine serum, 100 IU/mL penicillin-streptomycin, respectively, and incubated at 37 °C in a 5% CO_2/95% air humidified incubator. HeLa cells were grown in RPMI 1640 medium.

1.4 Preparation of telomerase extract from Hela cells

The telomerase extracts were prepared according to the previously reported protocol.[2] First, HeLa cells were collected in the exponential phase of growth and counted. Then 1×10^6 cells were transferred into an RNase-free 1.5 ml EP tube and washed twice with PBS by centrifugation at 2,000 rpm for 5 min. After discarding the supernatant carefully, the cells were resuspended in 200 μL of NP-40 lysis buffer (10 mM Tris-HCl, pH 8.0, 1 mM $MgCl_2$, 1 mM EDTA, 1% NP-40, 0.25 mM sodium deoxycholate, 10% glycerol, 150 mM NaCl, 5 mM β-mercaptoethanol, 0.1 mM AEBSF), and incubated for 30 min on ice, and then centrifuged at 12,000 rpm for 20 min at 4 °C. Finally, the supernatant was collected and transferred to microcentrifuge tube and stored at -80 °C until use. The telomerase extracts from other cells were prepared in the similar protocol. The telomerase extracts of Hela cell for inactivated Hela cell were heat-treated at 95 °C for 10 min according to the previously reported protocol.[S2]

1.5 Telomerase extension reaction

Telomerase extracts were first diluted with lysis buffer to 5 μL of a series of different concentrations, and then 20 μL of the telomerase extension reaction buffer (20 mM Tris-HC1, pH 8.3, 1.5 mM $MgC1_2$, 1 mM EGTA, 63 mM KCl, 0.05% Tween 20, 1 mM dNTPs and 2 μM TS primer) was added. The mixtures were then incubated at 30 °C for 2 h. For the control experiments, it was carried out with 0 cell telomerase extracts.

1.6 The signal amplification reaction

After the telomerase extension reaction, 5.0 μL H1 (2.0 μM), 20 U T7 exonuclease, 5 μL 10×NEBuffer 4, and appropriate amount of DEPC water were

added to the telomerase extension reaction system, which made a total volume of the reaction to be 50 μL. Then the mixtures were immediately tested after being incubated at 25 °C for 2 h.

1.7 Agarose gel electrophoresis

For the gel electrophoresis of the Telomerase extension reaction, 5 μL of telomerase extracts was added to 20 μL of the telomerase extension reaction buffer (20 mM Tris-HC1, pH 8.3, 1.5 mM $MgC1_2$, 1 mM EGTA, 63 mM KCl, 0.05% Tween 20, 1 mM dNTPs and 2 μM TS primer). After the mixture solution was incubated at 30 °C for 2 h, the products from telomerase-catalyzed elongation was analyzed by agarose gel (4%) electrophoresis. The electrophoresis condition was 100 V for 40 min in 0.5×TBE buffer.

For the gel electrophoresis of T7 exonuclease-aided signal amplification reaction, 5.0 μL T1 (2.0 μM), 5.0 μL H1 (2.0 μM), 20 U T7 exonuclease and 5 μL 10×NEBuffer 4 were mixed, and appropriate amount of DEPC water were added to above mixed solution to made a total volume of 50 μL. The mixtures were immediately tested by agarose gel (4%) electrophoresis after being incubated at 25 °C for 2 h. The electrophoresis condition was 100 V for 80 min in 0.5×TBE buffer.

1.8 Activation of microchip channels

Microchip channels were activated according to the previously reported protocol.[1] Before use, vacuum pump was used to wash the microchip channels with methanol, ultrapure water, 1 M NaOH solution and ultrapure water sequentially for 30 min, and then the chip was soaked overnight in ultrapure water. Before each sample injection, the microfluidic channel was rinsed sequentially with ultrapure water for 5 min, 1M NaOH solution for 10 min, ultrapure water for 5 min and electrophoresis buffer for 5 min. Finally, all reservoirs were filled with the electrophoretic buffer solution. Vacuum was applied to the reservoir BW in order to fill the separation channel with the electrophoretic buffer solution for MCE-CL assay.

1.9 Detection of telomerase activity

After T7 exonuclease-aided signal amplification reaction, an appropriate amount of reaction solution was analyzed by MCE-CL. Prior to electrophoresis, electrophoretic buffer solution in reservoir S was replaced by 10 μL reaction solution, then different voltages were applied to respective reservoirs to complete sample injection, separation and detection. During the sample injection period, the sample reservoir S was applied 400 V, SW reservoir 0 V, B reservoir 200 V, BW reservoir 250 V and R reservoir was floated. After 20 s, and potentials were switched to the separation and detection period immediately. During this period, S and SW reservoirs were applied 2000 V, B reservoir 2400 V, BW reservoir 0 V, R reservoir 500 V.

2. References

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3. Supplementary Tables and Figures

Table S1 Oligonucleotide sequences

Name	Sequence
TS primer	5'-AATCCGTCGAGCAGAGTT-3'
T1	5'-AATCCGTCGAGCAGAGTTAGGGTTAGGGTTAGGGTTAGGG-(FAM)-3'
H1	5'-(HRP)-CCCTAACCCTAACCCTAACCCTAACCCTAACCCTAA-3'
H2	5'-(FAM)-CCCTAACCCTAACCCTAACCCTAACCCTAACCCTAA-3'

Table S2. Comparison of the analytical performance for the detection of telomerase activity between the proposed method and methods reported in the literature

Strategy	Linearity range	Detection limit	Reference
Fluorescence based telomerase triggered DNA walker	152-12187 cells	90 HeLa cells	S3
Fluorescence based exonuclease-assisted target recycling amplification assay	5-100 cells	5 HeLa cells	S2
Exponential isothermal amplification of telomere repeat assay	1-200 cells	1 HeLa cell	S4
TRAP assay	1-10000 cells	1293 cells	S5
Au nanoparticle-based ECL assay	313-10000 cells	148 HL-60 cells	S6
ECL sensor based on porphyrin-functionalized graphene	10-750 cells	10 HeLa cells	S7
Colorimetry and SERS spectroscopy	10-1000 cells	10 SKBR3	S8
Heterogeneous electrocatalytic assay	100-10000 cells	100 A549	S9
Amperometry baesd nanoparticle-mediated signal amplification	1-1000 cells	1 MCF-7 cell	S10
Homogeneous electrochemical assay based on exonuclease-assisted target recycling amplification assay	2-1000 cells	1 HeLa cell	S11
Chemiluminescence amplification strategy	1-1000 cells	1 Hela cell	This work

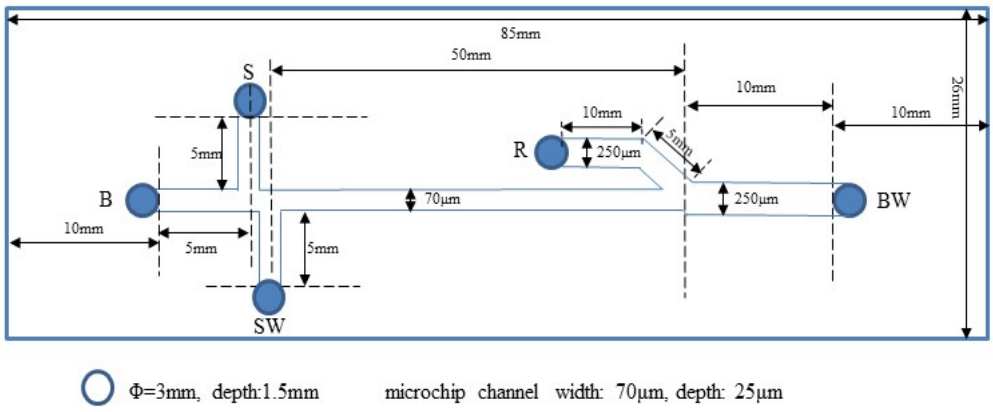

Fig. S1. The structure of microchip for MCE-CL

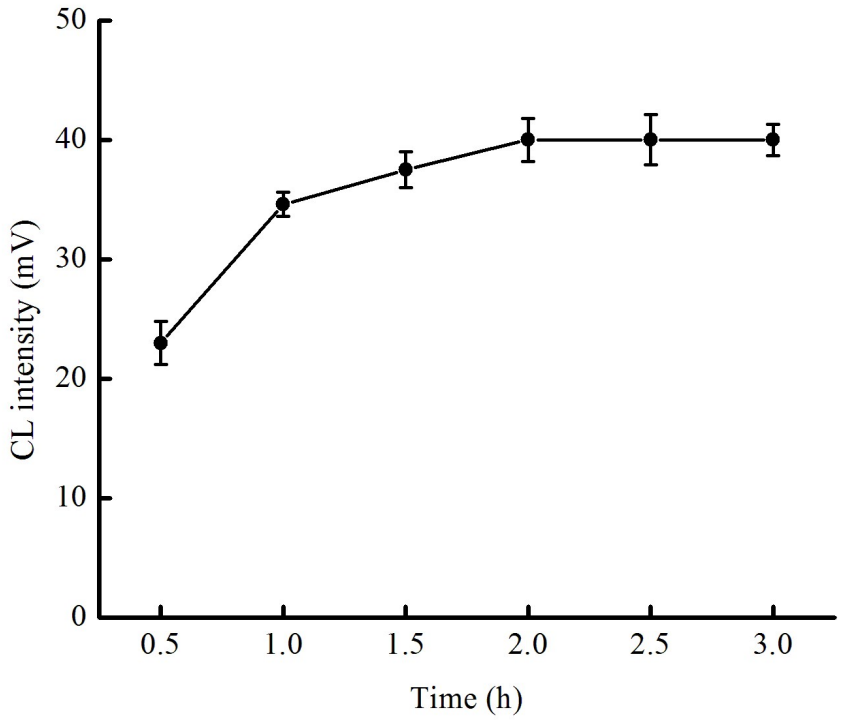

Fig. S2. The Effect of telomerase extension reaction time

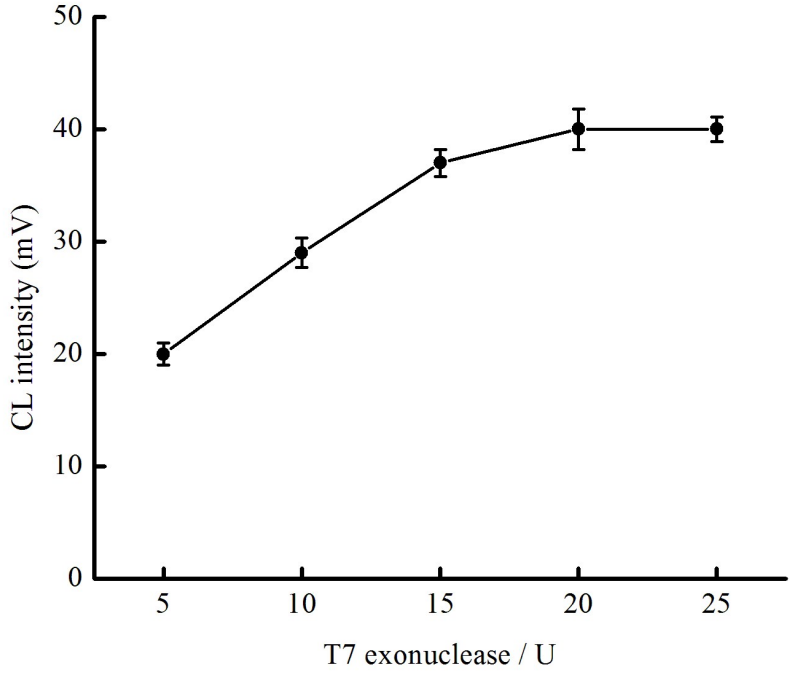

Fig. S3. The effect of the T7 exonuclease amount

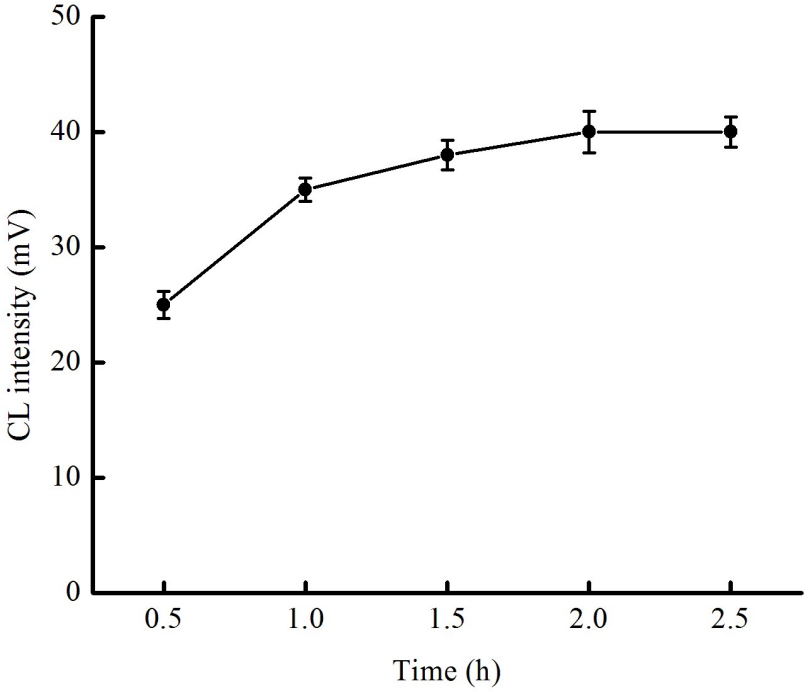

Fig. S4. The effect of the reaction time for T7 exonuclease

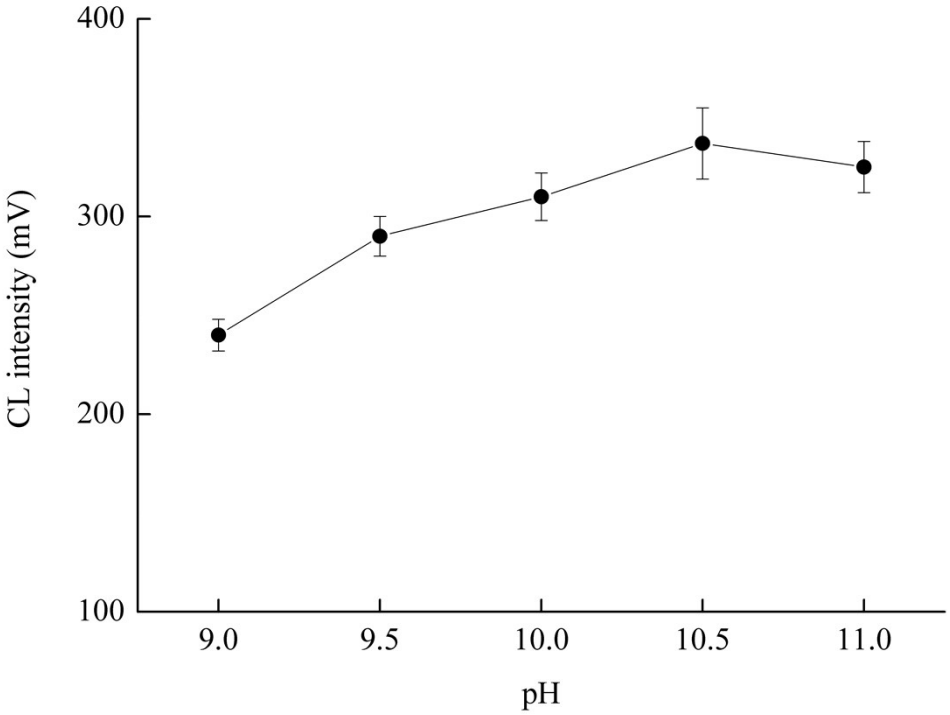

Fig. S5. The effect of the pH of oxidant solution

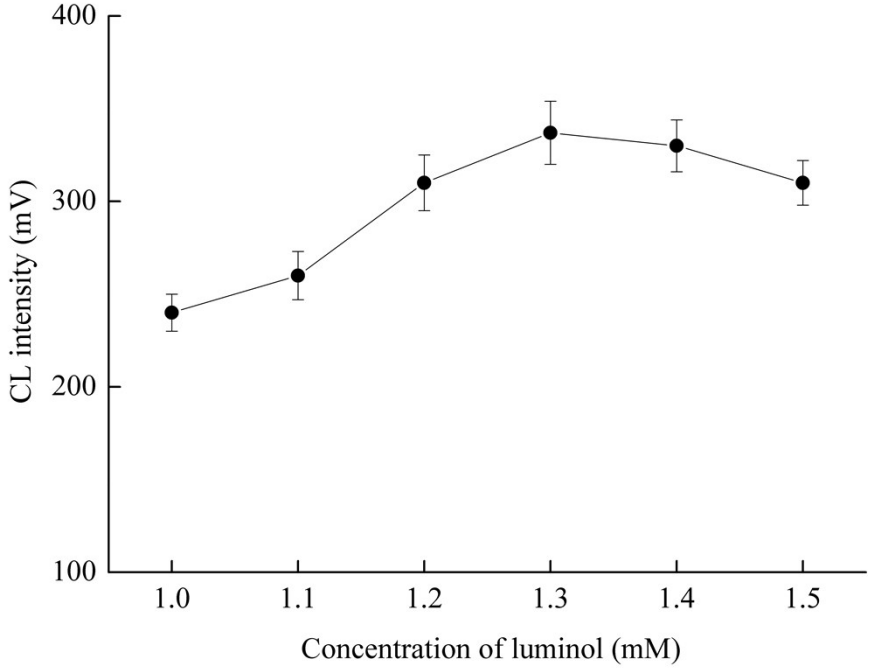

Fig. S6. The effect of the luminol concentration

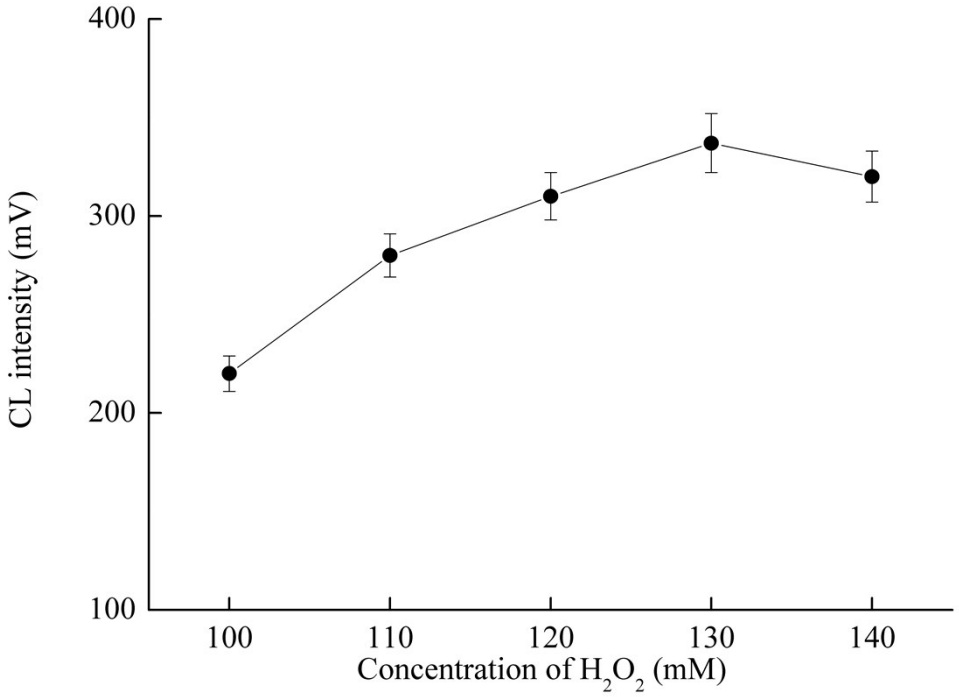

Fig. S7. The effect of the hydrogen peroxide concentration

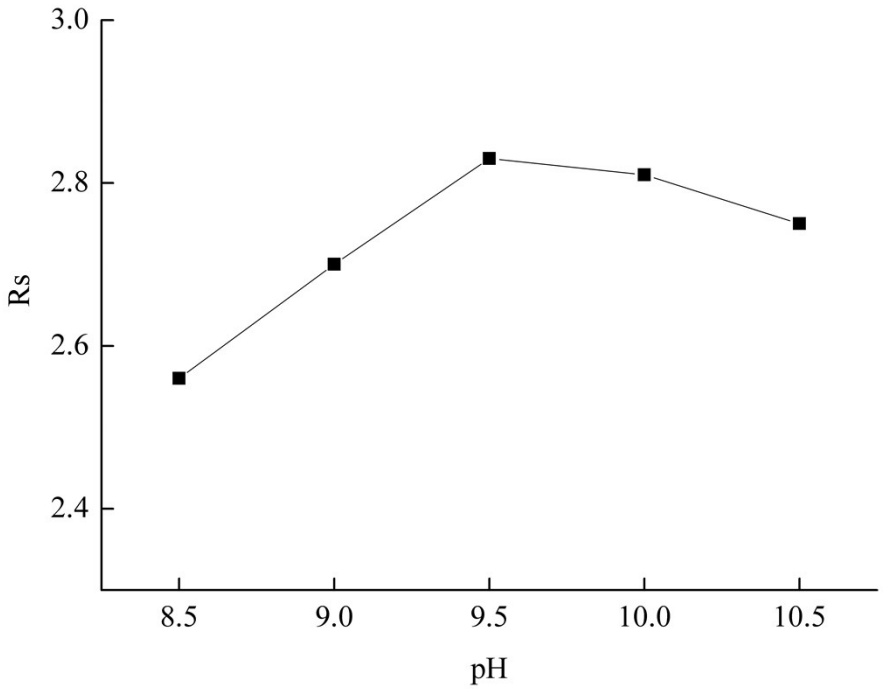

Fig. S8. The effect of the pH of electrophoresis buffer solution to the separation

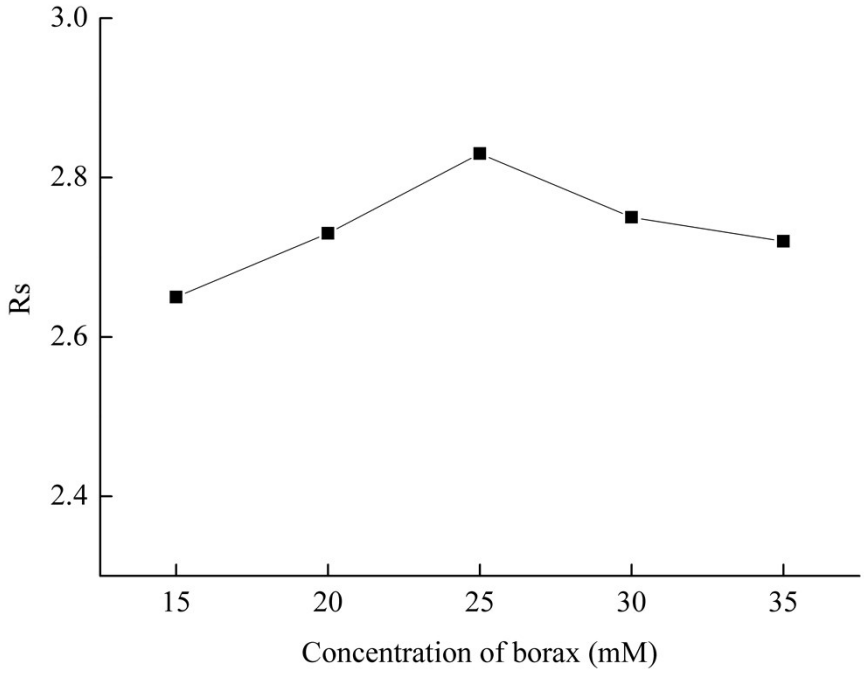

Fig. S9. The effect of the borax concentration to the separation

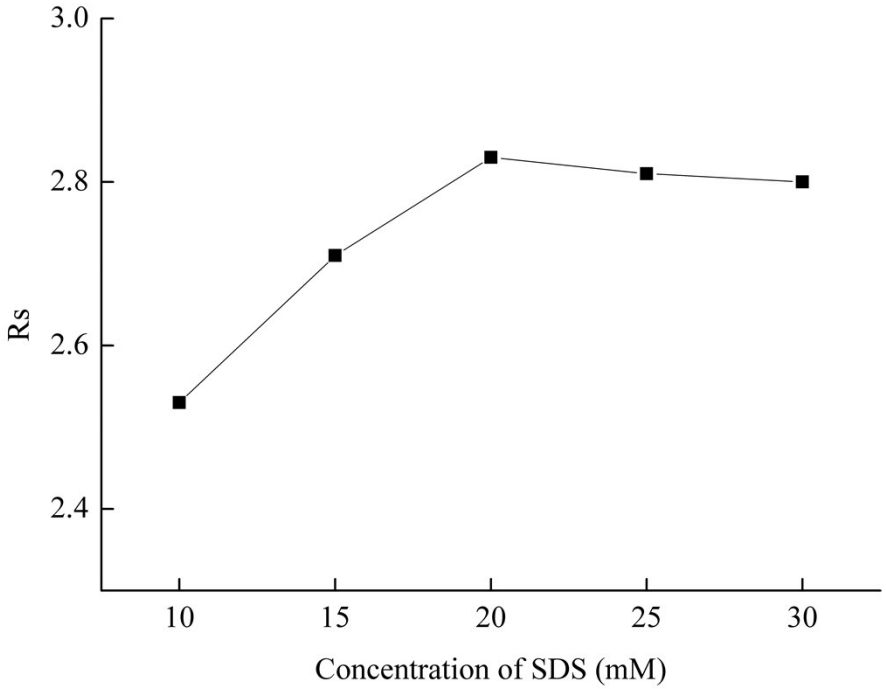

Fig. S10. The effect of the SDS concentration to the separation

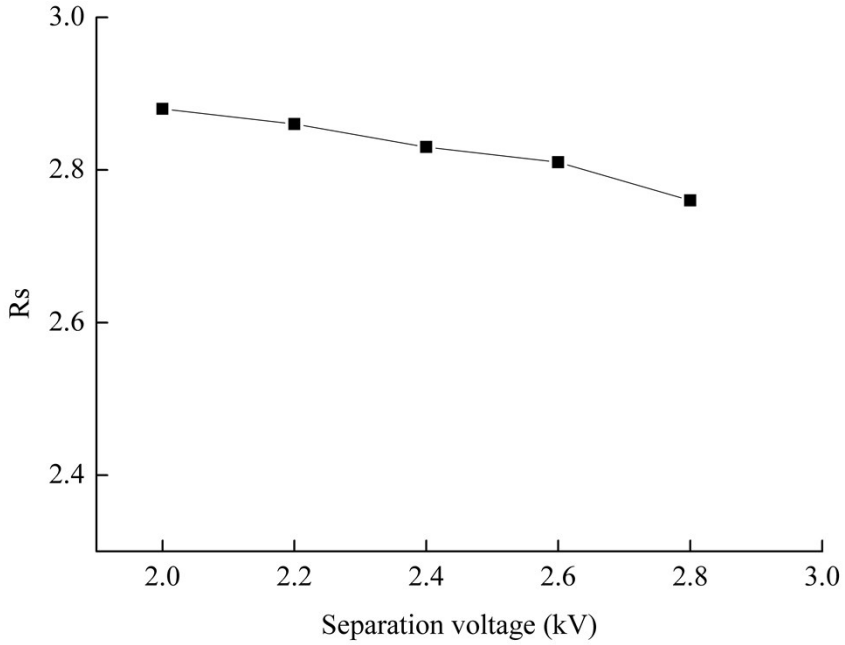

Fig. S11. The effect of separation voltage to the separation

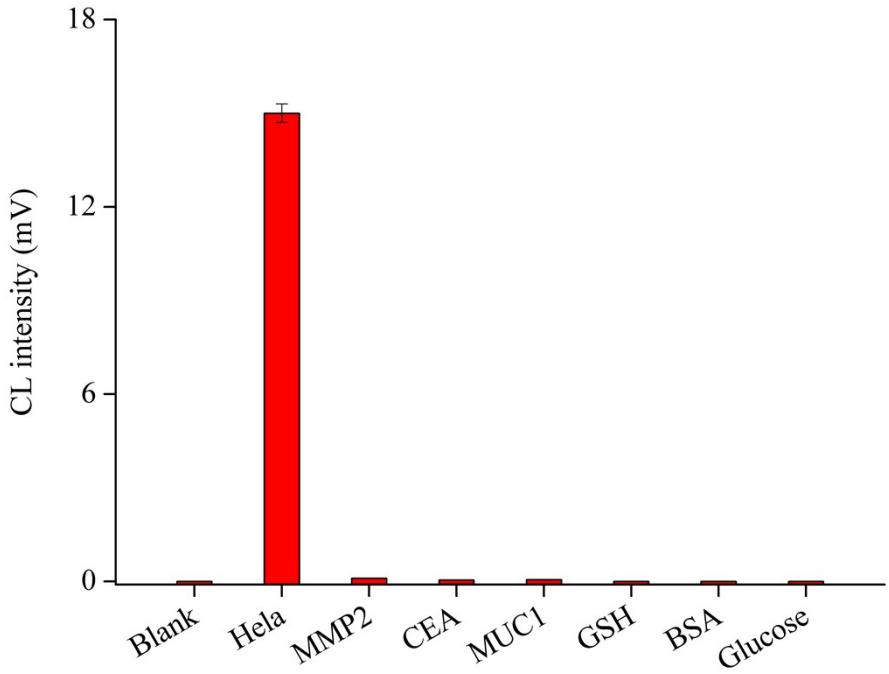

Fig. S12. Study on the specificity of the method. The concentration of substances is: blank, 0 cell; Hela, 200 cells; BSA, 100 μg/mL; GSH, 3 mg/mL; Glucose, 10 mg/mL; other substances: 10 μg/mL.