

Supporting Information:

Culture of the MCF-7 and L02 cell lines

MCF-7 cell lines were purchased from the Chinese Academy of Sciences (Shanghai, China), culturing in Minimum Eagle's Medium (MEM). The culture media was supplemented with 10% fetal bovine serum (Sigma-Aldrich, USA), 100 units/mL penicillin and 100 µg/mL streptomycin (Thermo Fisher). L02 cell lines were from laboratory save, culturing in 1640 medium (Hangzhou Jinuo Bio-pharmaceutical Tech. Co., Ltd., China). The culture media were supplemented with 10% fetal bovine serum (Sigma-Aldrich, USA). All of the cells were grown in an atmosphere of 5% CO₂ at 37 °C.

Synthesis of the circular probe

Circular probe was synthesized by ligation of the circle primer. 30 µL reaction solution containing 1 µM circle primer, 1 µM ligation primer, 1 × T4 DNA ligase buffer (66 mM Tris-HCl, 6.6 mM MgCl₂, 10 mM DTT, 0.1 mM ATP, pH 7.6) and 10 U/ µL T4 DNA ligase (Takara) was incubated at 16 °C for 2 h. Then, the circle primers that were not ligated and ligation primers were digested at 37 °C for 1 h by adding 10U Exonuclease I (Takara) and 100U Exonuclease III (Takara). Finally, denature the enzyme at 80 °C for 20 min.

Gel electrophoresis analysis

The 2% agarose gel electrophoresis was carried out in 0.5× Tris-acetate-EDTA (TAE) (Shanghai Sangon, China) at a constant voltage of 100 V for 30 min at room temperature. Nucleic acid dye Gene finder (Xiamen Zeesan Biotech. Co., Ltd., China) was added beforehand. Gels were photographed by gel image instrument (Xiamen Zeesan Biotech. Co., Ltd., China).

RCA assay for miR-21 detection without *fishhook* probe based magnetic separation

30 μ L amplification reaction solution containing 1 \times Phi 29 polymerase buffer (50 mM Tris-HCl, 10 mM MgCl₂, 10 mM (NH₄)₂SO₄, 4 mM DTT, pH 7.5), 200 μ M dNTP, 0.2 U/ μ L Phi 29 polymerase, 10 nM circular probe and different concentrations of miR-21 was incubated at 31 °C for 2 h. Then, 1.5 μ L 20 \times Evagreen Dye and 1.5 μ L EDTA (0.5 M, pH 8.0) was added and the fluorescence was recorded by the smartphone based imaging device.

Table S1. Sequences of nucleic acids used in the experiment

primer	sequence
3-biotin-immobilization	5-
probe	CAACCACACTGGCAAGAGGC AAAAAAAAAAAA AAAA-biotin
Circle primer for miR-21	5-p- GTGGTTGTCTTCT <u>TCAACATCAGTCTGATAAG</u> <u>CTAATAACATTATACGCCATCCTCAGCCAGCCT</u> CTTGCCAGT
Ligation primer	AGAAGACAACCACACTGGCAAGAGGC
miR-21	5- UAGCUUAUCAGACUGAUGUUGA
Let-7a	5- UGAGGUAGUAGGUUGUAUAGUU
miR-141	5- UAA CAC UGU CUG GUA AAG AUG G
miR-155	5- UUA AUG CUA AUC GUG AUA GGG GU
M1	5- UAGCUUAUCAUACUGAUGUUGA
M3	5- UAGCUGAUCAUACUGACGUUGA
M5	5- UAUCUGAUCAUACUGACGUUAA

Table S2. Comparison of present assay with reported methods for miRNA detection

Method	Real sample	Total RNA extraction	Detection technique	Instrumentation	Performance	References
CHA, RCA and ALP labeled signal amplification	Cultured cells	YES	Electrochemistry	Electrochemical workstation	0.5-12500 pM	<i>Analyst</i> , 2018, 143, 2304
Isothermal exponential amplification reaction (EXPAR)	Cultured cells	YES	Electrochemistry	Electrochemical workstation	20 fM-50 pM	<i>Anal. Chem.</i> 2014, 86, 8200
SDA, DNAzyme catalysis reaction and magnetic separation	Human serum	YES	Fluorescence	Fluorescence spectrophotometer	1 fM-50 pM	<i>Biosens. Bioelectron.</i> , 2017, 96, 106
DNAzyme-assisted target recycling, RCA and HRP-mimicking DNAzyme catalysis	Cultured cells	YES	Electrochemiluminescence	Electrochemiluminescence analyzer	1 fM-100 pM	<i>Anal. Chem.</i> 2015, 87, 3202
rGO based magnetic solid-phase extraction and RCA	Human plasma	YES	Fluorescence	RT cycler	100 aM-1 pM	<i>Anal. Chem.</i> 2017, 89, 10137
Magnetic separation and RCA	Cultured cells	NO	Fluorescence	Smartphone	100 fM-1 nM	Present work

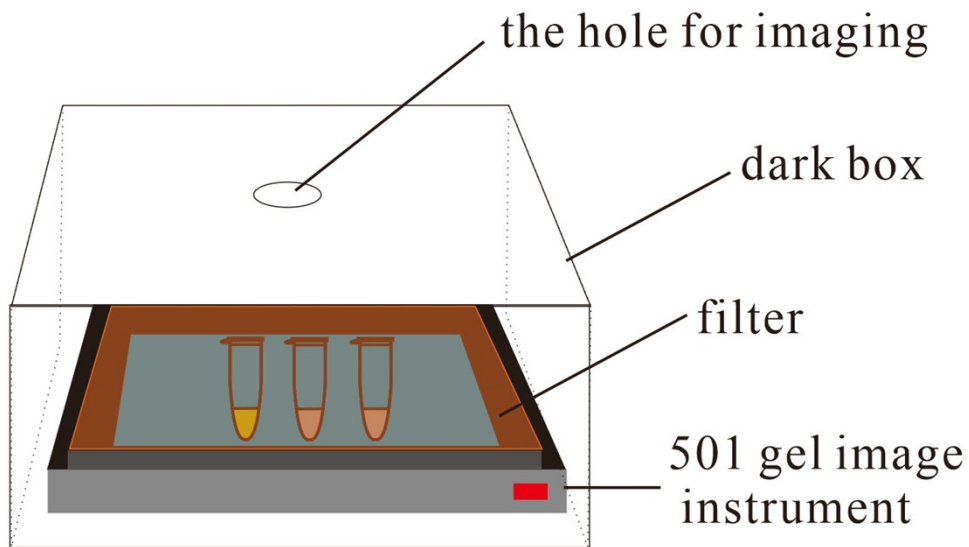


Figure S1. Smartphone based imaging device

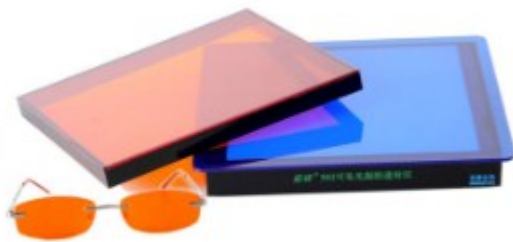


Figure S2. 501 Visible light gel image instrument

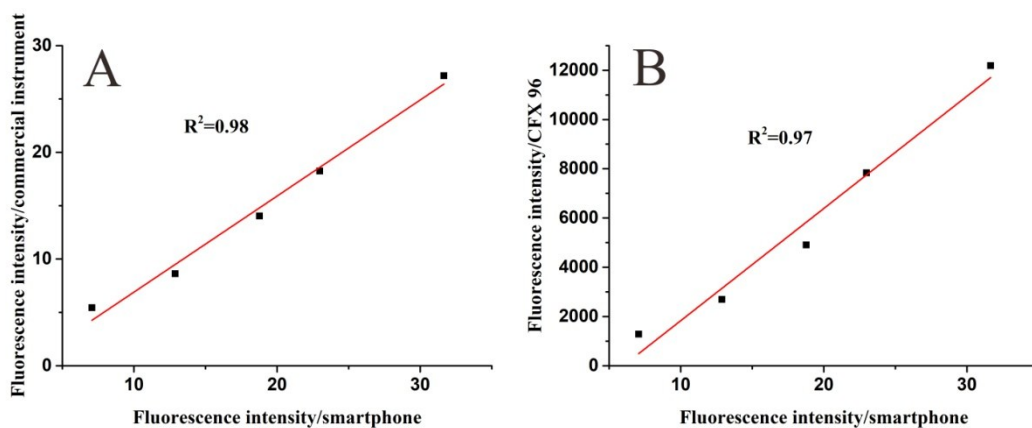


Figure S3. Comparison of the fluorescence intensity analysis based on smartphone and commercial 621 visible light gel image instrument (A); fluorescence quantitative analysis based on smartphone and CFX96 Touch real-time PCR detection system (25

°C, detection interval of 1 min) (B); concentration of fluorescein sodium is 50, 100, 200, 300, 500 nM in 10 mM sodium borate.

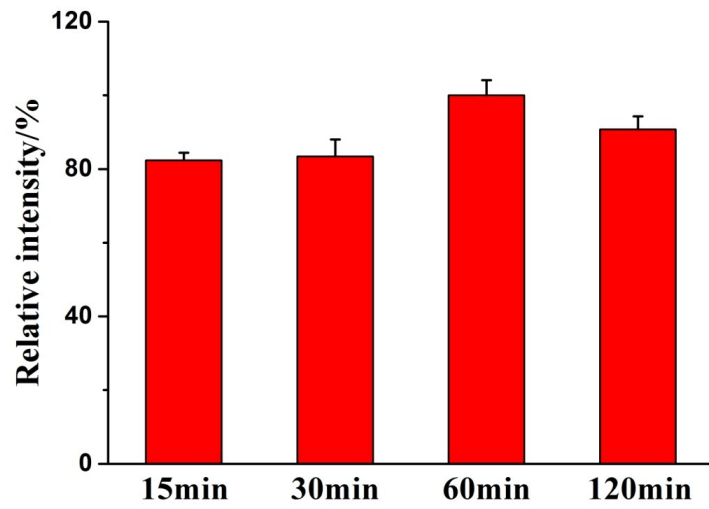


Figure S4. The influence of the hybridization time for miR-21 detection.

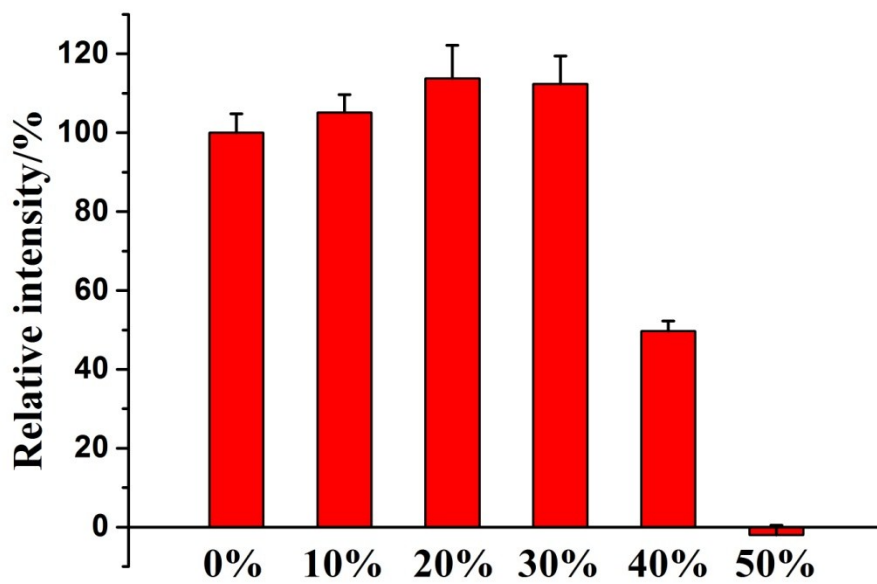


Figure S5. The influence of the formamide concentration for miR-21 detection when washing after hybridization process (formamide concentration refers to the volume fraction in 1× PBS).

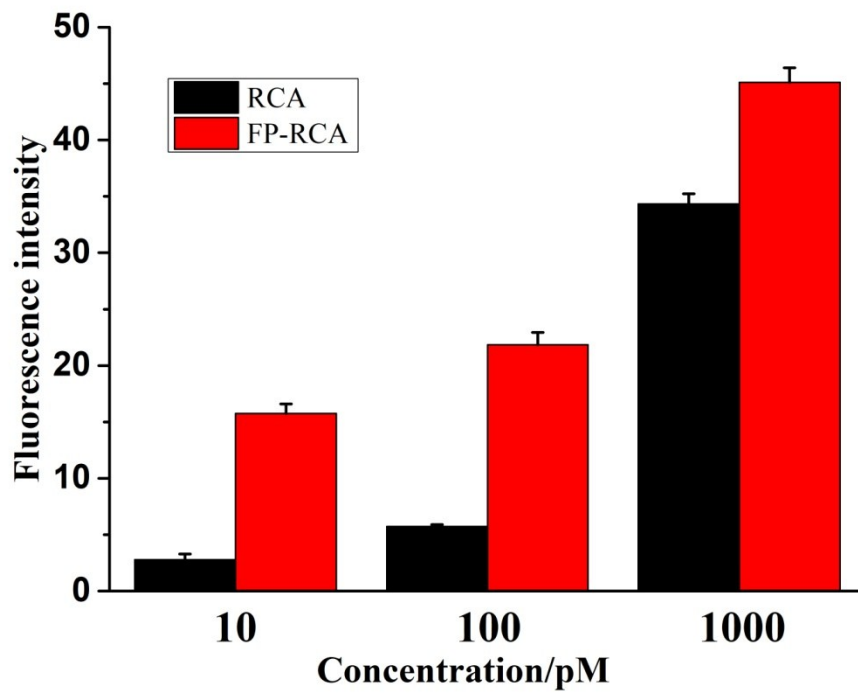


Figure S6. The fluorescence intensity in the presence of different concentrations miR-21 with RCA and FP-RCA assay.