

Electronic Supplementary Information

A label-free aptamer-based biosensor for microRNA detection by the RNA-regulated fluorescence of malachite green

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1. Materials and apparatus

Bst DNA polymerase large fragment (8 U/ μ L), T7 RNA polymerase (50 U/ μ L) and 10 \times T7 RNA polymerase reaction buffer (40 mM Tris-HCl, 2 mM spermidine, 6 mM MgCl₂, 10 mM dithiothreitol, pH 7.9 @ 25°C) were purchased from New England Biolabs (USA). Malachite Green (MG) was purchased from Sigma-Aldrich. The oligonucleotides, dNTPs, NTPs, RNase inhibitor, 20 bp DNA ladder, 6 \times nucleic acid sample loading buffer and RNase-free water were obtained from TaKaRa Biotechnology Co. Ltd. (Dalian, China). Potassium chloride (KCl), Magnesium chloride (MgCl₂), Sodium chloride (NaCl), 4S Red Plus nucleic acid stain, Trihydroxymethyl aminomethane (Tris) were obtained from Sangon Biotech Co. Ltd. (Shanghai, China). The A549 was purchased from the cell bank of Chinese Academy of Sciences (Shanghai, China). All the reagents were of analytical grade and were used as received without further purification. The miRNAs, MG RNA aptamer (MGA), and extension DNA template were purified by HPLC and their sequences were listed in Table S1:

The fluorescence spectra were obtained by F-7000 fluorescence spectrometer (Hitachi). The imaging of polyacrylamide gel electrophoresis (PAGE) was performed by using the Gel DocTM EZ Imager (Bio-Rad). The reaction temperature was controlled by 2720 thermal cycle system (Applied Biosystems, USA). The RNA was stored in Forma ultra-low temperature freezer (Thermo Scientific, USA).

Table S1. The oligonucleotides were used in this work.

Name	Sequence (5'-3')
let-7a	UGAGGUAGUAGGUUGUAUAGUU
let-7b	UGAGGUAGUAGGUUGUGUGGUU
let-7c	UGAGGUAGUAGGUUGUAUGGUU
let-7d	UGAGGUAGUAGGUUGCAUAGUU
let-7e	UGAGGUAGGAGGUUGUAUAGUU
let-7f	UGAGGUAGUAGAUUGUAUAGUU
let-7g	UGAGGUAGUAGUUUGUACAGUU
let-7i	UGAGGUAGUAGUUUGUGCUGUU
MGA	GGAUCCCGACUGGCGAGAGCCAGGUAACGAAUGGAUCC
Extension	GGATCCATTCGTTACCTGGCTCTCGCCAGTCGGGATCCTC
DNA	TCCCTATAGTGAGTCGTATTAACCTATAACAACCTACTACC
template	TCA

2. Cell culture and total RNA extraction

The A549 cell line was cultured in 5 mL F-12K Medium (Invitrogen, Cat. 21127022) containing 10% (v/v) fetal bovine serum. The cells were maintained at 37 °C under a humidified atmosphere containing 5% CO₂.

When the bottom of the culture bottle was filled with cells, the Medium was removed from the culture bottle, and the cells were washed three times with PBS (10 mM sodium phosphate buffer, 0.1 M NaCl, pH 7.4 @25 °C). Then, isolation procedures of total RNA from cells were according to the instructions of the TRIZOL[®] Reagent (Invitrogen). Finally, the concentrations of total RNA extracts were quantified using the NanoDrop One. The total RNA extracts were stored in Forma ultra-low temperature freezer (Thermo Scientific, USA).

3. Standard protocols of microRNA assay

The reaction mixtures of the label-free aptamer-based biosensor for microRNAs detection were prepared separately as Part A and Part B, and operated on ice.

Part A consisted of 1.0 μL 10 \times T7 RNA polymerase reaction buffer, 1.0 μL 1 μM extension template, 0.2 μL 40 U/ μL RNase inhibitor, 1.0 μL target microRNA, and 1.8 μL RNase-free water. Part B consisted of 1.0 μL 50 U/ μL T7 RNA polymerase, 1.0 μL 8 U/ μL Bst DNA polymerase large fragment, 1.0 μL 2.5 mM dNTPs, 1.0 μL 10 mM NTPs, and 1.0 μL RNase-free water.

Part A and part B were mixed evenly. The mixed solution was placed in 2720 thermal cycle system, and incubated at 37 $^{\circ}\text{C}$ for 2 hours to complete the extension reaction. After the extension reaction is completed, the mixed solution is heated to 75 $^{\circ}\text{C}$ for 10 minutes and then cooled room temperature.

4.0 μL 100 μM MG and 186 μL Tris-HCl buffer (20 mM Tris, 5 mM NaCl, 5 mM MgCl_2 , 140 mM KCl, pH=7.4) were added to the above reaction solution and fluorescence spectra were scanned from 630 nm to 700 nm by using F-7000 fluorescence spectrometer.

4. Non-denaturing polyacrylamide gel electrophoresis (PAGE) analysis

Non-denaturing polyacrylamide gel electrophoresis (PAGE) was used to confirm the reaction products of miRNA assay. The reaction products (10 μL) mixed with 6 \times loading buffer (2 μL) was loaded on to a 16% non-denaturing PAGE gel. The PAGE experiments were performed in 1 \times TBE (90 mM Tris, 90 mM Boric acid, 2 mM EDTA, pH=8.3 @ 25 $^{\circ}\text{C}$) buffer at 110 V for 80 min. Subsequently, the gel was stained by 2 \times 4S Red Plus Nucleic Acid Stain for 5 min in 1 \times TBE buffer. Finally, the gel was imaged by using Gel DocTM EZ Imager.