Supporting Information for

All-in-one detection of breast cancer-derived exosomal miRNA on pen-based paper chip

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Preparation of lateral flow strip

i. Pretreatment of Sample Pads

Immerse a 20 cm × 30 cm glass fiber membrane (GL-b04) in 50 mL of sample pad treatment solution. Air-dry at room temperature overnight and then dry in a 37 ℃ oven for 4 hours. After drying, cut into 2 cm × 30 cm strips, seal in aluminum foil bags, and store in a constant temperature and humidity chamber for later use.

ii. Preparation of Detection Probes (AuNPs-FAM-Antibody)

Take 1.5 mL of 13 nm colloidal gold solution, add 0.2 M K_2CO_3 to adjust the pH to 9.0. The mixture was thoroughly shaken and mixed well. Add 20 μg of streptavidin and react at room temperature for 2 hours. Add 10 μL of 10% BSA, incubate at room temperature for 1 hour, then centrifuge (10 min, 4 ℃, 10000 rpm). The supernatant liquid was discarded while the labeled complex precipitate obtained after resuspending with a volume of 100 μL served as the detection probe (AuNPs-FAM-Antibody), which were stored away from light at 4 ℃ until further use.

iii. Preparation of Conjugate Pads

Cut a 22 cm × 30 cm glass fiber membrane (8964) into 1 cm × 30 cm strips and set aside in a constant temperature and humidity chamber. Spray the detection probe onto the 1 cm × 30 cm glass fiber membrane at a rate of 6 μL/cm. Air-dry overnight in a 37 ℃ oven, seal in aluminum foil bags, and store in a constant temperature and humidity chamber for later use.

iv. Preparation of Reaction Membrane

In the direction from conjugate pad to absorbent pad, coat 1 mg/mL anti-FAM antibody and 1 mg/mL biotin using a spray-coating instrument at a rate of 1 μL/cm onto nitrocellulose membrane (NC membrane), designated as the test line and control line, respectively. After drying overnight in a 37 ℃ convection oven, seal in aluminum foil bags and store in a constant temperature and humidity chamber for later use.

v. Preparation of Absorbent Pads

Cut a 20 cm × 30 cm absorbent paper (H-2) into 2.5 cm × 30 cm strips and set aside in a constant temperature and humidity chamber.

vi. Assembly of Test Strips

Assemble all components on a PVC baseplate in the following order: sample pad, conjugate pad, reaction membrane, and absorbent pad. Each section should overlap by 1-2 mm. Use a programmable KM-3100 strip cutter to cut the assembled strip into a width of 4 mm. Place the cut strips into aluminum foil bags, seal with desiccant, and store in a constant temperature and humidity chamber for later use.

Table S1 Existing methods for microRNAs detection

Reference	Platform	Target	Detection Method	Mechanism	Limits of detection
Li et al. [1]	Electrochemical biosensor	Lytic Exosome/ miRNA-21	Electrochemical biosensor	Primer exchange reaction with MOF@Pt@MOF nanozyme	0.29 fM
Li et al. [2]	Carbon Nanotube Field-Effect Transistor Biosensor	Purified exosomal miRNA/ miRNA-21	Carbon Nanotube Field-Effect Transistor Biosensor	Hybridization between the immobilized DNA probe and target miRNA	0.87 aM
Yang et al. [3]	Micromixer Biochip	Intact Exosome/ miRNA-21 TTF-1 mRNA	Fluorescence-based	Cationic Lipoplex Nanoparticles	2.06 × 10^9 and 3.71 × 10^9 exosomes /mL
Zhao et al. [4]	Thermophoresis	Intact Exosome/ miRNA-375	Fluorescence-based	Hybridization of DNA probes by nanoflares	0.36 fM
Cho et al. [5]	96-well plates	Intact Exosome/ miRNA-21 miRNA -574-3p CD63 EpCAM	Fluorescence-based	Molecular beacon	-
Deng et al. [6]	Lateral flow assay	Lytic tumor cells/ miRNA-21	Quantum dots-labeled strip biosensor	Enzyme-free catalyzed hairpin assembly	200 aM
Qian et al. [7]	Agarose-based microfluidic chip	Intact Exosome/ miRNA-21	Fluorescence-based	Enzyme-free catalyzed hairpin assembly	1000 exosomes
This work	Pen-based paper chip	Lytic Exosome/ miRNA-21	Lateral flow assay	Enzyme-free catalyzed hairpin assembly	25 fM

Table S2 Sequences of DNA probes and microRNA

Name	Sequences (5'-3')
HP1	1 2 3 4* 3* 2* TCAACATC-AGTCTGA-TAAGCTA-CCATGTGTAGA-TAGCTTA-TCAGACT-FAM
HP2	3 4 3* 2* 4* TAAGCTA-TCTACACATGG-TAGCTTA-TCAGACT-CCATGTGTAGA-Biotin
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-210	CUGUGCGUGUGACAGCGGCUGA
miR-214	ACAGCAGGCACAGACAGGCAGU

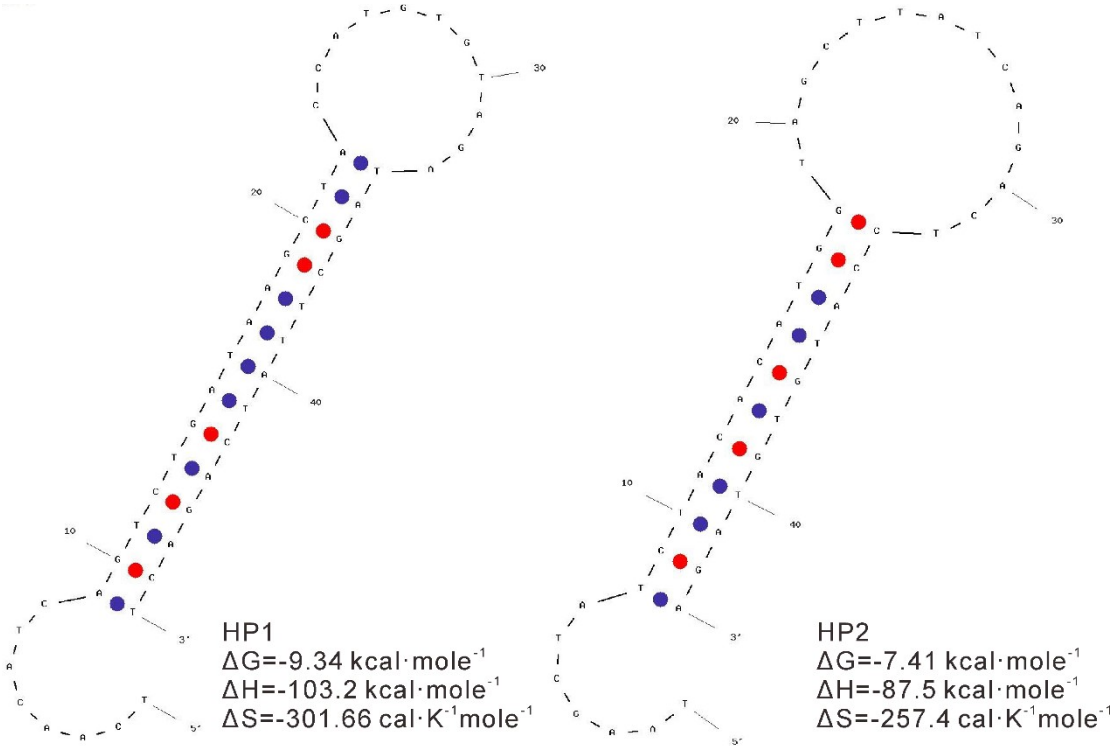

Figure S1. Structure image and thermal kinetic parameters of HP1 probe and HP2 probe.

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