

Electronic Supplementary Information (ESI) for:

**A versatile magnetic beads-based flow cytometric assay
for detection of thyroid cancer related hsa-miR-221-3p in
blood and tissue**

Jiaxue Gao,^{ab} Jia Wei,^c Yaoqi Wang,^c Zhenshengnan Li,^c Lina Ma,^{*a} Xianying
Meng ^{*c} and Zhenxin Wang ^a

^aState Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, 130022, P. R. China.

^bUniversity of the Chinese Academy of Sciences, Beijing, 100049, P. R. China.

^cDepartment of Thyroid Surgery, The First Hospital of Jilin University, Changchun, Jilin 130021, P. R. China.

*Corresponding author:

E-mail: malina@ciac.ac.cn (L.M.), mengxiany@jlu.edu.cn (X.M.).

Tel/Fax.: +86431-85262757 (L.M.).

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1. Supplementary Experimental Section

1.1 qRT-PCR analysis of different cell lines

Total RNAs of TPC-1 and Nthy-ori 3-1 cell lines were extracted according to the miRNA extraction kit (Qiagen Co., Inc., USA) instructions. To measure the expression levels of has-miR-221-3p, total RNAs were transcribed by stem-loop reverse transcription (RT) primer using BioTeke super RT Kit (BioTeke Co., Beijing, China). Quantitative PCR (qPCR) were performed using Hieff UNICON® Universal Blue qPCR SYBR Green Master Mix (Yeasen Bio. Ltd., Shanghai, China) on ABI QuantStudio 5 system using the protocol provided by Yeasen. U6 levels were used as an internal control. The primers are listed as follows:

Reverse transcription primer of has-miR-221-3p:

GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGATACGACGAAAC
CCA

qPCR primers :

has-miR-221-3p: forward primer: GCCGAGAGCTACATTGTCTG; reverse
primer: GTCGTATCCAGTGCAGGG

U6: forward primer: CTCGCTTCGGCAGCACA; reverse primer:
AACGCTTCACGAATTTGCGT

2. Additional Table S1-S5

Table S1. Sequences of used oligonucleotides in the experiments.

Name	Sequence (5' to 3')
DNA probe	Biotin-TTTTTTTTTTGAACCCAGCAGACAATGTAGCT
hsa-miR-221-3p	AGCUACAUUGUCUGCUGGGUUUC
hsa-miR-221-5p	ACCUGGCAUACAAUGUAGAUUU
hsa-miR-222-3p	AGCUACAUCUGGCUACUGGU
hsa-miR-222-5p	CUCAGUAGCCAGUGUAGAUCU
hsa-miR-let-7a	UGAGGUAGUAGGUUGUAUAGUU
hsa-miR-let-7b	UGAGGUAGUAGGUUGUGUGGUU
hsa-miR-let-7c	UGAGGUAGUAGGUUGUAUGGUU
NC-miRNA	UUCUCCGAACGUGUCACGUTT

Table S2. Comparison between the proposed MBs-FCM assay and other reported methods for miRNA detection.

Analytical method ^a	Dynamic range	Detection limit	References
FRET	0 nM - 10 nM	60 pM	[1]
Electrochemical	1 nM - 50 nM	28.1 nM	[2]
Chemiresistive	10 pM - 100 nM	14.6 pM	[3]
Fluorescence	0.4 nM - 4 nM	0.2 nM	[4]
Colorimetric	0.5 pM -1 nM	0.5 pM	[5]
Fluorescence	0.02 nM - 100 nM	4 pM	[6]
FCM	0.01 nM -10 nM	2.1 pM	This work

^a FRET, Fluorescent resonance energy transfer; FCM, flow cytometry.

Table S3. Recoveries for the spiked synthetic hsa-miR-221-3p in four human plasma obtained by this assay (n=3).

Samples	Spiked (pM)	Measured (pM)	RSD (%)	Recovery (%)
Sample 1 (PTC)	5	5.49	3.1	109.8
	50	54.78	4.7	104.8
	500	532.62	2.6	106.5
Sample 3 (PTC)	5	5.37	3.5	107.4
	50	53.68	1.4	107.3
	500	547.83	3.9	109.5
Sample 3 (nodule)	5	4.64	4.2	92.8
	50	48.68	1.4	97.3
	500	524.69	1.9	104.9
Sample 4 (nodule)	5	4.73	3.7	94.6
	50	54.36	4.6	108.6
	500	538.72	2.8	107.7

Table S4. The information of 27 PTC patients.

Patients Number	Gender	Age	Diagnosis	TNM Tumor Staging
1	female	44	PTC	IIIA (T1aN1aM0)
2	female	52	PTC	IIIA (T1aN1aM0)
3	female	59	PTC	IB (T1bN0M0)
4	female	30	PTC	IA (T1aN0M0)
5	female	55	PTC	IIIA (T1aN1aM0)
6	male	40	PTC	IIIB (T1bN1bM0)
7	female	45	PTC	IIIA (T1aN1aM0)
8	female	28	PTC	IIIB (T1bN1bM0)
9	male	46	PTC	IA (T1aN0M0)
10	female	32	PTC	IIIA (T1aN1aM0)
11	female	48	PTC	IA (T1aN0M0)
12	female	46	PTC	IIIA (T1bN1aM0)
13	male	26	PTC	IIIA (T2N1aM0)
14	female	50	PTC	IIIA (T1bN1aM0)
15	female	58	PTC	IIIA (T1aN1aM0)
16	female	28	PTC	IB (T1bN0M0)
17	male	26	PTC	IB (T1bN0M0)
18	female	52	PTC	IA (T1aN0M0)
19	female	25	PTC	IIIB (T2N1bM0)
20	female	55	PTC	IA (T1aN0M0)
21	male	49	PTC	IIIA (T1aN1aM0)
22	female	53	PTC	IIIA (T1aN1aM0)
23	female	34	PTC	IA (T1aN0M0)
24	female	44	PTC	IIIA (T1bN1aM0)
25	female	47	PTC	IA (T1aN0M0)
26	male	36	PTC	IIIA (T1aN1aM0)
27	male	79	PTC	IIIB (T1bN1bM0)

Table S5. Comparison of the detection results by this assay to ultrasonography results.

Patients Number	Our assay ^a	Ultrasonography results
1	C	C
2	N	N
3	N	N
4	N	N
5	N	C
6	C	C
7	C	C
8	N	C
9	N	N
10	N	C
11	C	C
12	C	C
13	C	N
14	C	C
15	C	C

^a C represent cancer patient, N represent normal patient.

3. Additional Figure S1-S5

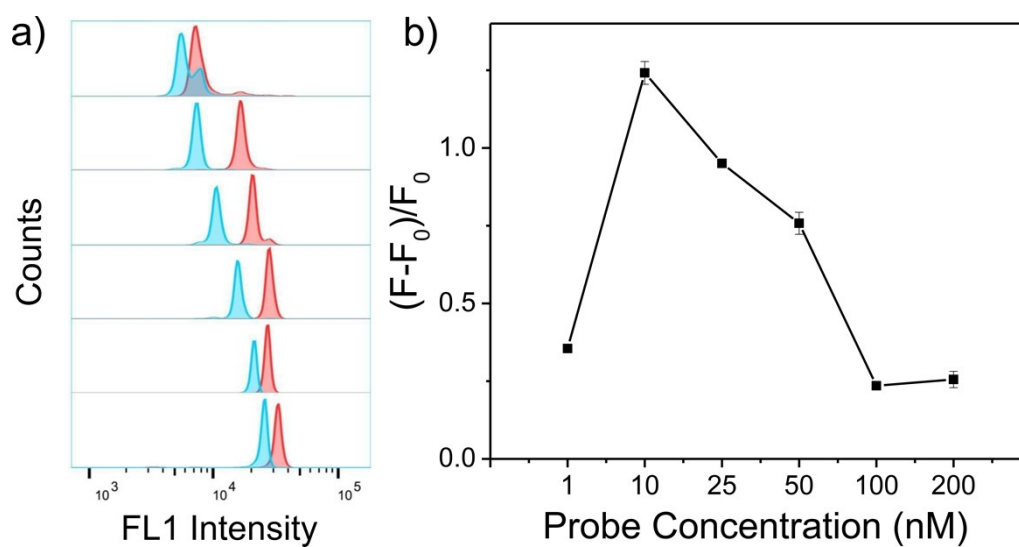


Fig. S1. Effect of the probe densities of the MBs-probe conjugates for the detection of has-miR-221-3p. a) Fluorescence responses of the MBs-probe conjugates treated with 1 nM has-miR-221-3p (red lines) in comparison with blank control (blue lines) under different probe concentrations from up to down (1, 10, 25, 50, 100 and 200 nM). b) The corresponding relative fluorescence intensity changes with different probe concentrations. Error bars mean standard deviations (n=3).

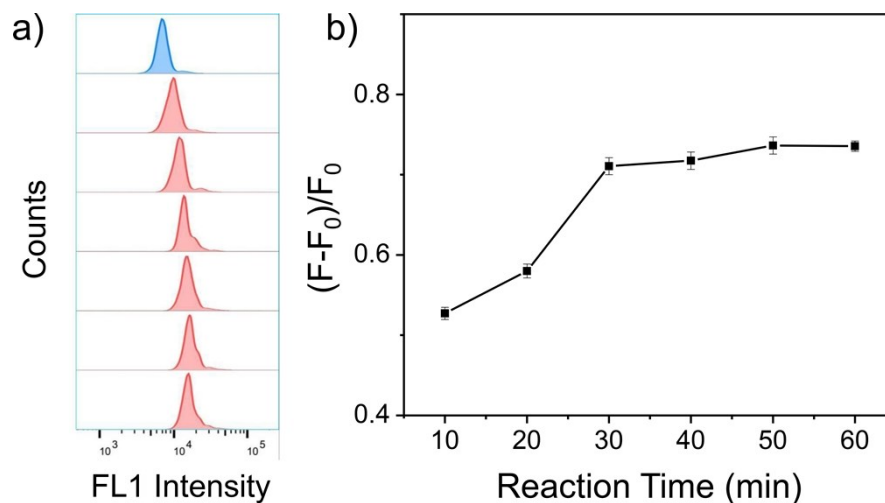


Fig. S2. Optimization of reaction time for the detection of hsa-miR-221-3p. a) Fluorescence responses of the MBs-probe conjugates treated with 100 pM hsa-miR-221-3p (red lines) in comparison with blank control (blue line) under different reaction time from up to down (10, 20, 30, 40, 50 and 60 min). b) The corresponding relative fluorescence intensity changes with different reaction times. Error bars mean standard deviations (n=3).

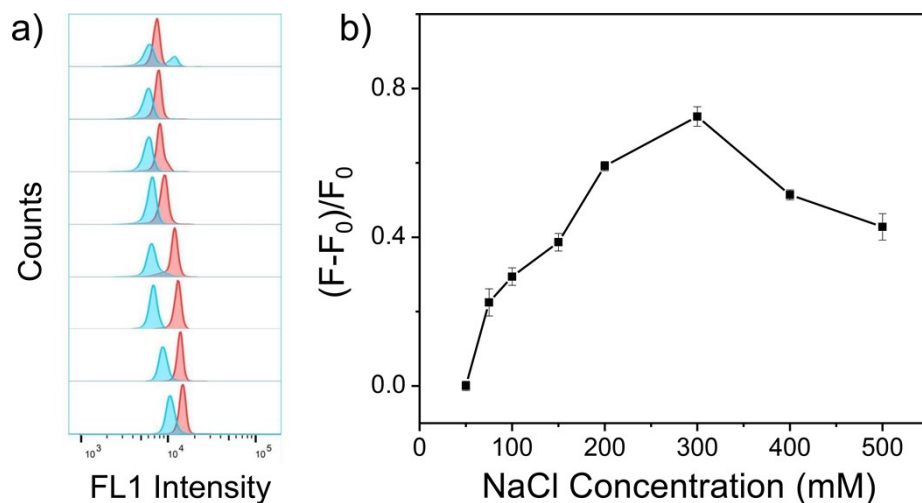


Fig. S3. Effect of NaCl concentration for the detection of hsa-miR-221-3p. a) Fluorescence responses of the MBs-probe conjugates treated with 100 pM hsa-miR-221-3p (red lines) in comparison with blank control (blue lines) under different concentrations of NaCl from up to down (50, 75, 100, 150, 200, 300, 400 and 500 mM). b) The corresponding relative fluorescence intensity changes with different concentrations of NaCl. Error bars mean standard deviations (n=3).

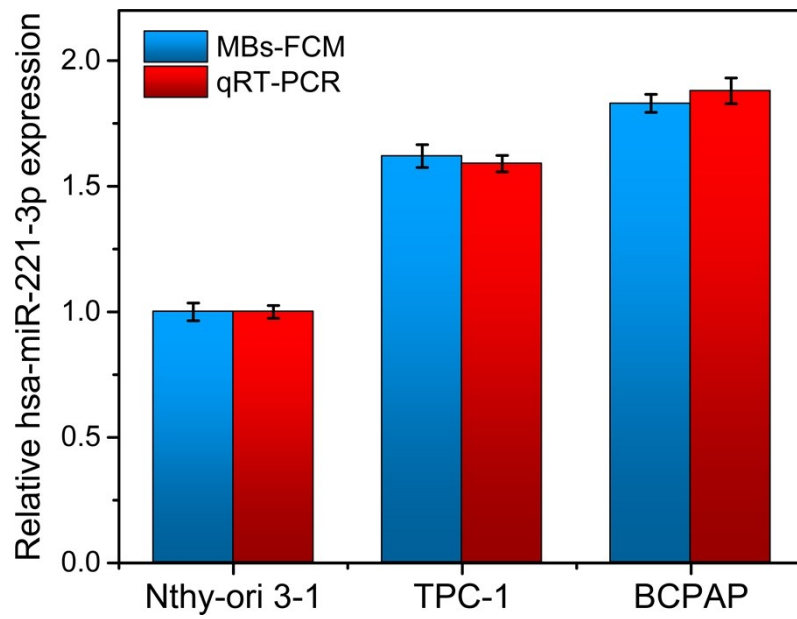


Fig. S4. Relative expression levels of has-miR-221-3p in TPC-1, BCPAC and Nthy-ori 3-1 cell lines obtained by the proposed MBs-FCM assay and qRT-PCR. Error bars mean standard deviations (n=3).

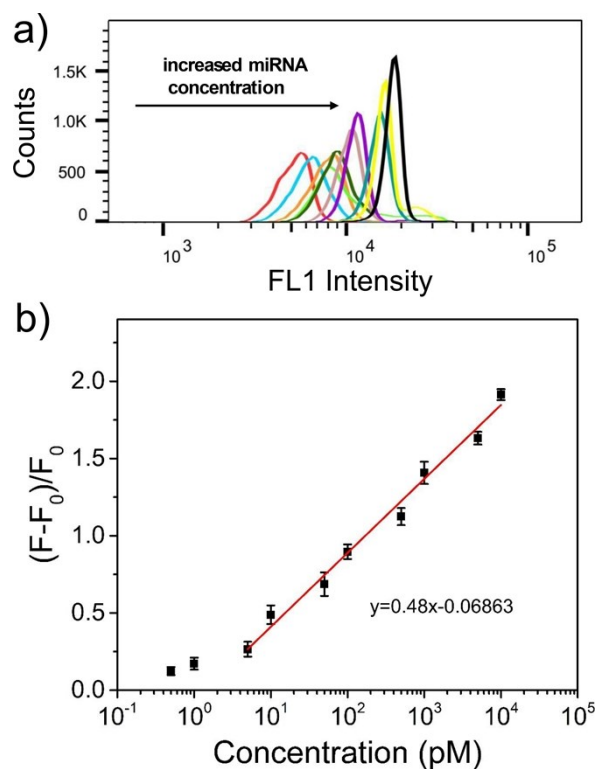


Fig. S5. a) Fluorescence response of MBs-probe conjugates incubated with 10% plasma spiked with different concentrations of hsa-miR-221-3p. b) The corresponding relative fluorescence intensity changes as a function of concentration of hsa-miR-221-3p. Error bars mean standard deviations (n=3).

4. Reference

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