Supplementary Information

A flux-adaptable pump-free microfluidics based self-contained platform for multiplex cancer biomarker detection

Bo Dai,†[a] Cheng Yin,†[a] Jiandong Wu,†[b] Wei Li,[a] Lulu Zheng,[a] Francis Lin,[c] Xiaodian Han,[d] Yongfeng Fu,*[e] Dawei Zhang,*[a] and Songlin Zhuang[a]

a. Engineering Research Center of Optical Instrument and System, the Ministry of Education, Shanghai Key Laboratory of Modern Optical System, University of Shanghai for Science and Technology, Shanghai, 200093, China.

b. Bionic Sensing and Intelligence Center, Institute of Biomedical and Health Engineering, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

c. Department of Physics and Astronomy, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada.

d. Department of Laboratory Medicine, Shanghai Cancer Center, Fudan University, Shanghai, 200032, China.

e. Department of Medical Microbiology and Parasitology, School of Basic Medical Sciences, Fudan University, Shanghai, 200032, China.

* Corresponding Authors: Yongfeng Fu, yffu@fudan.edu.cn; Dawei Zhang, dwzhang@usst.edu.cn

† The authors contributed equally to this work.

Supplementary Note

Fabrication of the pump-free microfluidic chip

The fabrication procedure of the pump-free microfluidic chip is illustrated in Fig. S1. A mold for the microchannels was firstly fabricated by photolithography technique. The pattern of the microchannels was designed by AutoCAD (Autodesk, Inc.) and printed on a film as a transparency mask. Negative photoresist (SU8-2025, MicroChem, Corp.) was spun on a 4-inch silicon wafer at a spinning rate of 3000 rpm for 30 seconds. Then, the photoresist was exposed under UV light through the mask for 4.5 seconds. The unexposed photoresist was removed in SU-8 developer (MicroChem, Corp.). After that, a replica of the microchannels was produced. Polydimethylsiloxane (PDMS) (Silgard 184 Elastomer Kit, Dow Corning) was prepared with the weight ratio of 10:1 1 (elastomer versus curing agent). PDMS was used to cast the pattern of the microchannels. The PDMS was solidified at 80 ºC for 4 hours and peeled off the mold. Two 8 mm-diameter holes were punched at the one end of the microchannels. Meanwhile, 0.5 μL of 0.2 mg/mL monoclonal antibody in protein spotting buffer A (CapitalBio Technology, China) for each biomarker was coated on the glass substrate (OPPolymerSlide™ D, CapitalBio Technology, China) by using CapitalBio SmartArrayer™ 136 (CapitalBio Technology, China). The substrate was then soaked in the 5% bovine serum albumin/phosphate buffered saline for 1 hour at room temperature. Finally, the PDMS was placed on the glass substrate with the teardrop-shaped units aligned with the coating area and the outlet hung out over the edge of the substrate.

Establishment of the standard curves and calculation of the concentration

In the preliminary experiment, the standard curves for carcinoembryonic antigen (CEA), Alpha-fetoprotein (AFP), carbohydrate antigen 125 (CA125) and carbohydrate antigen 19-9 (CA19-9) biomarkers are established by measuring the standard antigens of gradient concentration. Once the intensity for the standards is obtained, the relation between the intensity and the concentration can be described based on the four-parameter logistic (4PL) equation, *i.e.*

$$I = \frac{A - D}{1 + (x/C)^{B}} + D \tag{1}$$

where x is the concentration of the antigen, y the intensity response, A is the minimum absorbance, D is the maximum absorbance, C is the point of inflection and B is the slop factor of the curve at the inflection point. The response for the CEA, AFP, CA125 and CA19-9 standards is listed as follows.

$$I_{CEA} = \frac{256.042}{1 + (x/70.152)^{-1.087}} + 1.353 \tag{2}$$

$$I_{AFP} = \frac{136.865}{1 + (x/41.472)^{-1.626}} + 5.524 \tag{3}$$

$$I_{CA125} = \frac{236.537}{1 + (x/73.211)^{-1.319}} + 4.453 \quad (4)$$

$$I_{CA19-9} = \frac{239.547}{1 + (x/69.925)^{-1.510}} + 10.647 \quad (5)$$

In the testing of the unknown samples, the concentration can be calculated using the inverse functions as follows.

$$x = C\left(\frac{A - I}{I - D}\right)^{1/B} \quad (6)$$

Evaluation of the limit of detection

The limit of detection of the assays for CEA, AFP, CA125 and CA19-9 is evaluated in the preliminary experiment. The limit of detection is calculated as follows.

$$LOD = C\left(\frac{A - D}{I_{Blank} + 3\sigma_{Blank} - D} - 1\right)^{1/B} \quad (6)$$

where A, B, C, and D, are the parameters used in 4PL equation, I_{Blank} and σ_{Blank} are the average response and the standard deviation of the background intensity collected from the blank units.

Supplementary Videos

Video S1 Assay procedure for detecting multiplex cancer biomarkers.

Video S2 The investigation of the capillary suction of the filter paper. 20 μL of plasma, chemiluminescent peroxidase substrate and PBST were dripped on three pieces of filter paper respectively. The spreading diameter was measured after 30 seconds.

Supplementary Figures

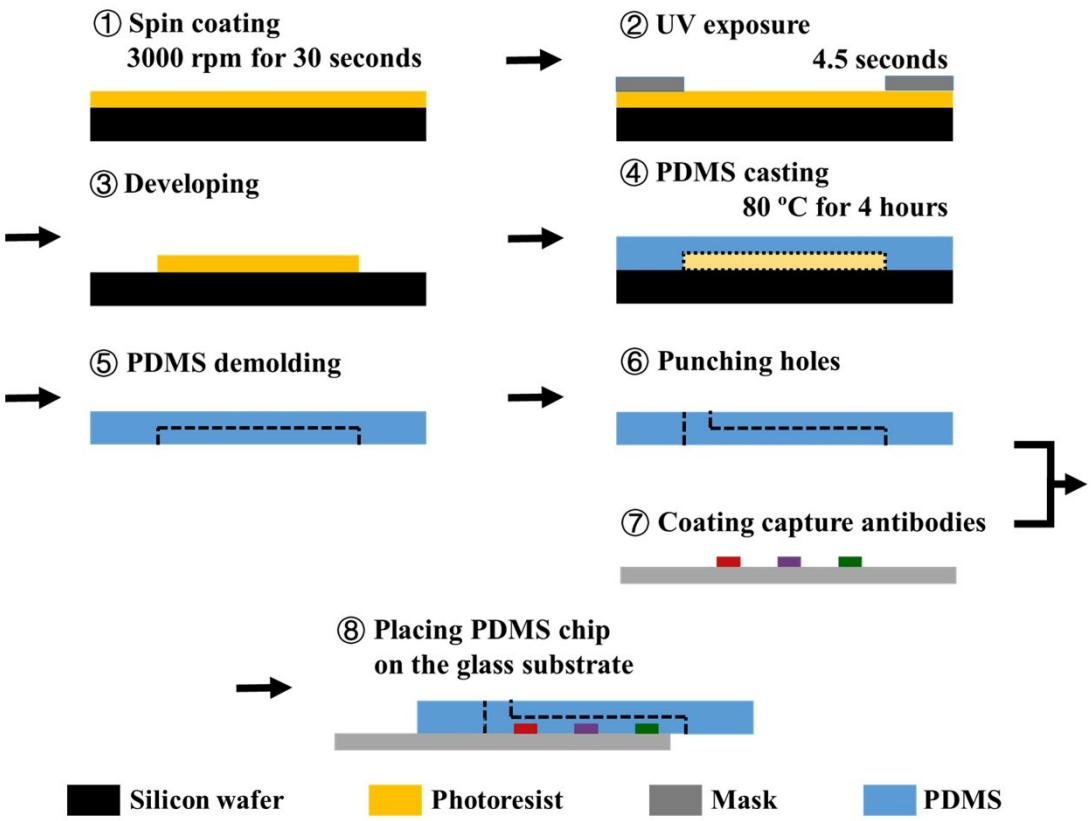

Figure S1 The fabrication process of the pump-free microfluidic chip.

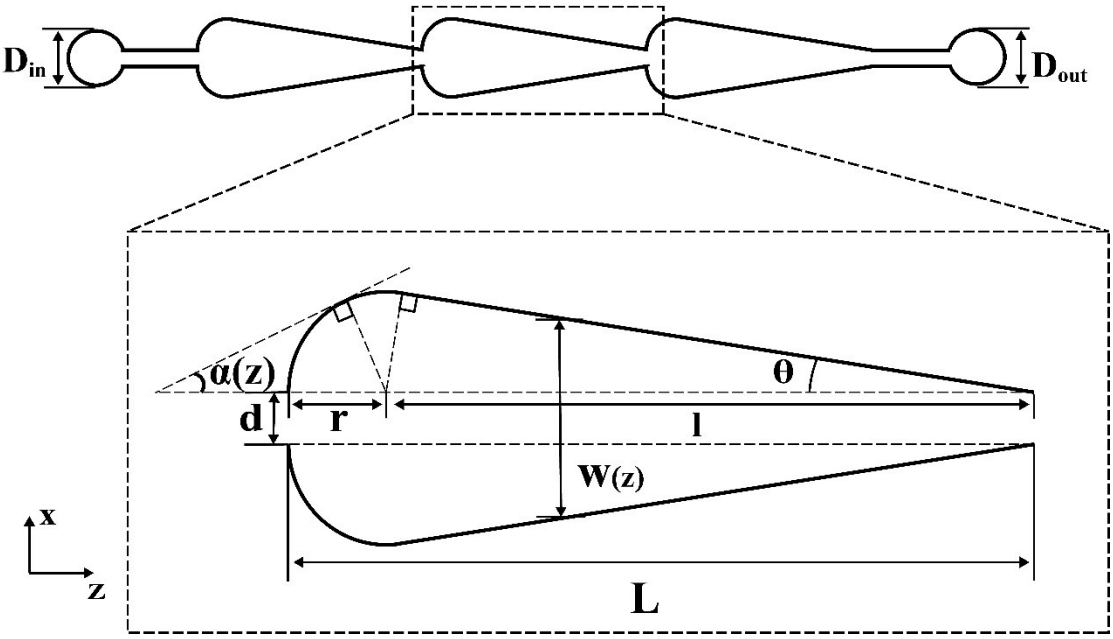

Figure S2 A structural model of the microchannel.

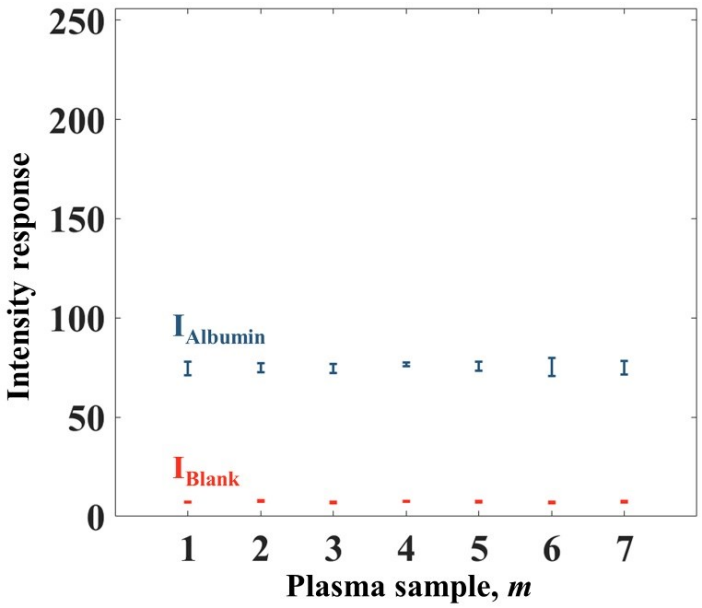

Figure S3 In the preliminary experiment, the intensity response of albumin and background for seven plasma samples is measured in the microfluidic chip by the SAP. The error bar represents the deviation of the results obtained in the three parallel experiments. $I_{Albumin\_Control}$ is an average value of the intensity response of albumin, $I_{Albumin}$, deducting the intensity of the background, I_{Blank}, i.e. $I_{Albumin\_Control} = \sum_{m=1}^{7} (I_{Albumin(m)} - I_{Blank(m)})/7$, where m is the sample number. Based on the measurement in the preliminary experiment, $I_{Albumin\_Control}$ = 67.84. $I_{Albumin\_Control}$ is used as a control value in the calibration of the intensity response of a specific cancer biomarker in Equation (1).

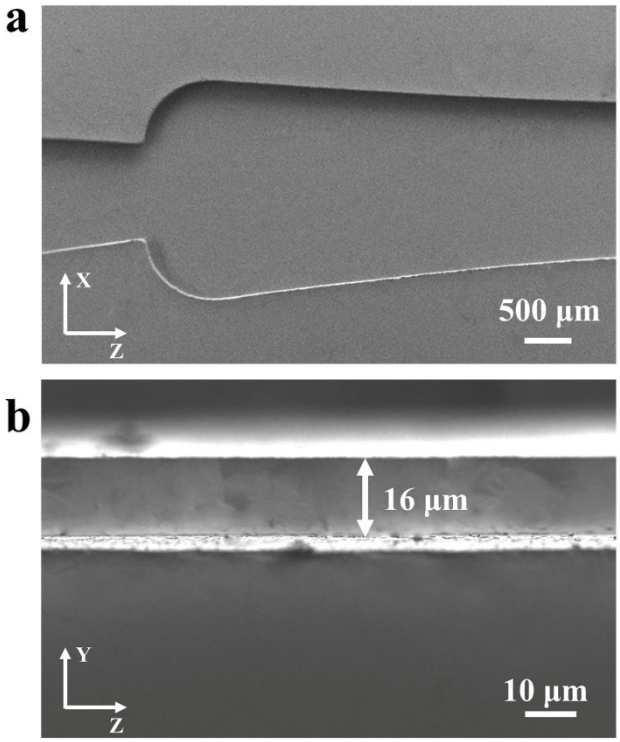

Figure S4 Scanning electron microscope (SEM) images of the microfluidic chip which is used in the flux analysis and the clinical testing. (a) The junction of the two units. (b) The side wall of the microchannel. The image was taken after the chip was cut along the flow direction.

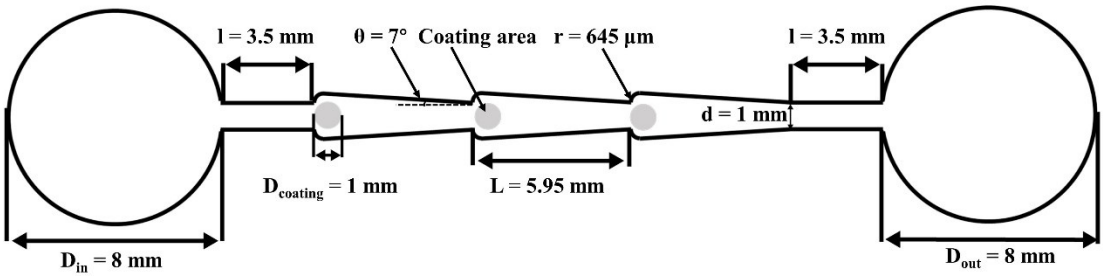

Figure S5 Dimensions of the microfluidic chip used in the flux analysis and the clinical testing. The height of the chip is 16 µm, as shown in Fig. S4.

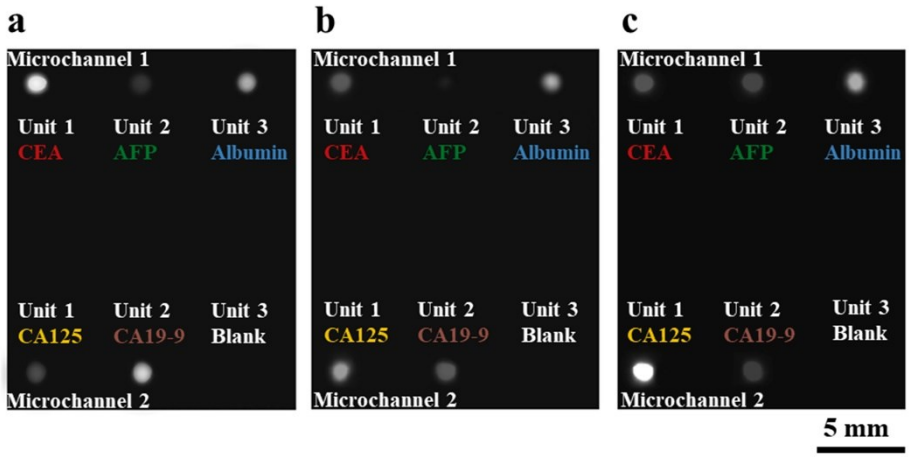

Figure S6 Images of the reaction products for three plasma samples in the pump-free microfluidic chip obtained by the SAP.

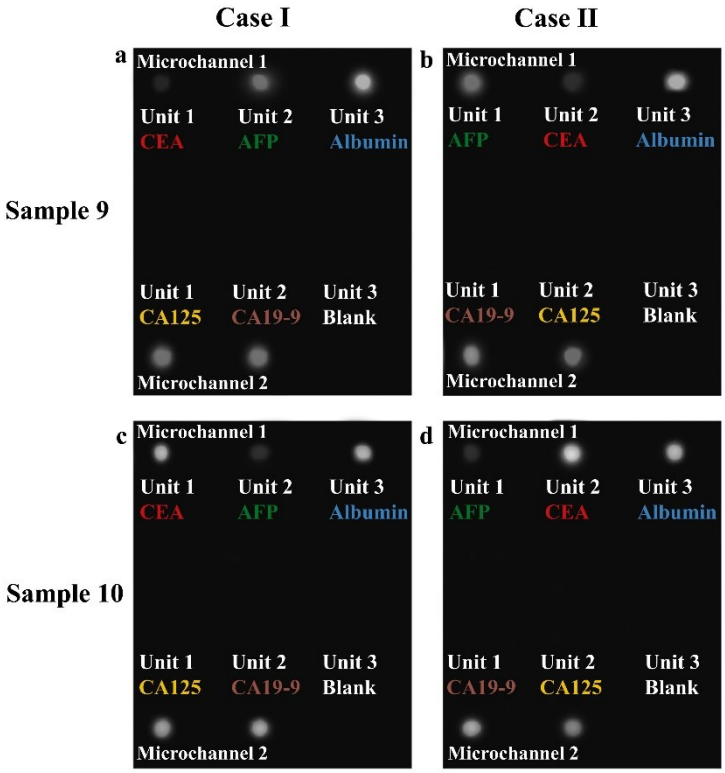

Figure S7 Images of the reaction products for the two plasma samples tested in the pump-free microfluidic chips using different coating strategies. Case I: capture antibodies for CEA and CA125 were immobilized in the first unit of the each microchannel and capture antibodies for AFP and CA19-9 were immobilized in the second unit of the each microchannel. Case II: capture antibodies for AFP and CA19-9 were immobilized in the first unit of the each microchannel and capture antibodies for CEA and CA125 were immobilized in the second unit of the each microchannel.

Table S1 The calculation of the concentration from the raw data and the comparison with those from the clinical testing. $I_{Albumin\_Control}$ =67.84. Difference percentage = $2(x - x_{Clinical})/(x + x_{Clinical}) \times 100\%$

Sample	Biomarker	The measured intensity of the biomarkers $I_{Biomarker}$	The measured intensity of the albumin $I_{Albumin}$	The measured intensity of the blank I_{Blank}	The calibrated intensity $I_{Biomarker\_Calib}$	The measured concentration x	The concentration from the clinical testing $x_{Clinical}$	Difference percentage
Sample 1	CEA	10.91	75.24	7.10	3.80	0.98 ng/mL	1.02 ng/mL	4.0%
	AFP	14.42			7.29	2.88 ng/mL	2.72 ng/mL	5.7%
	CA125	30.19			22.99	11.30 U/mL	10.70 U/mL	5.5%
	CA19-9	18.35			11.20	1.26 U/mL	1.2 U/mL	4.9%
Sample 2	CEA	17.45	74.34	7.54	10.07	3.23 ng/mL	3.06 ng/mL	5.4%
	AFP	13.79			6.35	1.79 ng/mL	1.72 ng/mL	4.0%
	CA125	20.51			13.17	6.17 U/mL	6.55 U/mL	6.0%
	CA19-9	54.29			47.48	22.60 U/mL	23.40 U/mL	3.5%
Sample 3	CEA	23.66	75.25	7.43	16.23	5.41 ng/mL	5.65 ng/mL	4.3%
	AFP	13.39			5.96	1.21 ng/mL	1.14 ng/mL	6.0%
	CA125	34.94			27.52	13.55 U/mL	14.40 U/mL	6.1%
	CA19-9	248.94			241.58	617.3 U/mL	636 U/mL	3.0%
Sample 4	CEA	21.99	76.43	7.32	14.40	4.76 ng/mL	4.97 ng/mL	4.3%
	AFP	17.14			9.64	4.90 ng/mL	5.01 ng/mL	2.2%
	CA125	83.08			74.37	37.90 U/mL	39.20 U/mL	3.4%
	CA19-9	249.85			238.08	487.50 U/mL	476.00 U/mL	2.4%
Sample 5	CEA	22.55	75.34	7.35	15.16	5.03 ng/mL	5.33 ng/mL	5.8%
	AFP	13.52			6.15	1.52 ng/mL	1.61 ng/mL	5.8%
	CA125	67.73			60.25	30.03 U/mL	28.30 U/mL	5.9%
	CA19-9	236.70			228.84	325.90 U/mL	335.00 U/mL	2.8%

Table S2 Investigation of the reproducibility of the assays.

Biomarker	Sample	The measured concentration *x*	Standard deviation
CEA	Sample 6	Test 1: 14.57 ng/mL Test 2: 14.64 ng/mL Test 3: 14.79 ng/mL	0.013
	Sample 7	Test 1: 1.67 ng/mL Test 2: 1.58 ng/mL Test 3: 1.77 ng/mL	0.009
	Sample 8	Test 1: 2.39 ng/mL Test 2: 2.57 ng/mL Test 3: 2.76 ng/mL	0.034
AFP	Sample 6	Test 1: 2.26 ng/mL Test 2: 2.33 ng/mL Test 3: 2.44 ng/mL	0.008
	Sample 7	Test 1: 4.72 ng/mL Test 2: 4.53 ng/mL Test 3: 4.64 ng/mL	0.009
	Sample 8	Test 1: 3.84 ng/mL Test 2: 3.64 ng/mL Test 3: 3.53 ng/mL	0.025
CA125	Sample 6	Test 1: 45.89 U/mL Test 2: 45.78 U/mL Test 3: 45.98 U/mL	0.010
	Sample 7	Test 1: 33.54 U/mL Test 2: 33.78 U/mL Test 3: 33.42 U/mL	0.034
	Sample 8	Test 1: 21.13 U/mL Test 2: 21.36 U/mL Test 3: 21.33 U/mL	0.016
CA19-9	Sample 6	Test 1: 92.74 U/mL Test 2: 92.62 U/mL Test 3: 92.43 U/mL	0.024
	Sample 7	Test 1: 13.94 U/mL Test 2: 14.24 U/mL Test 3: 13.93 U/mL	0.031
	Sample 8	Test 1: 36.53 U/mL Test 2: 36.52 U/mL Test 3: 36.31 U/mL	0.015

Table S3 Evaluation of the influence of the coating sequence over assay performance

Sample	Case	Microchannel	Unit	Biomarker	The measured concentration *x*
Sample 9	Case I	1	1	CEA	1.49 ng/mL
			2	AFP	11.37 ng/mL
		2	1	CA125	23.64 U/mL
			2	CA19-9	8.76 U/mL
	Case II	1	1	AFP	11.29 ng/mL
			2	CEA	1.52 ng/mL
		2	1	CA19-9	9.03 U/mL
			2	CA125	24.90 U/mL
Sample 10	Case I	1	1	CEA	41.21 ng/mL
			2	AFP	2.69 ng/mL
		2	1	CA125	67.96 U/mL
			2	CA19-9	20.83 U/mL
	Case II	1	1	AFP	2.78 ng/mL
			2	CEA	40.28 ng/mL
		2	1	CA19-9	20.36 U/mL
			2	CA125	68.09 U/mL

Table S4 The assay cost per sample testing for the quantitative multiplex detection of the four cancer biomarkers

Reagent and consumables (Manufacturer)	**Price (US$)**	**Total amount**	**Amount/ testing**	**Cost/ testing (US$)**
Mouse anti-CEA monoclonal antibody (BiosPacific Inc., USA)	357.00	1 mg	0.1 μg	0.04
Mouse anti-AFP monoclonal antibody (BiosPacific Inc., USA)	357.00	1 mg	0.1 μg	0.04
Mouse anti-CA125 monoclonal antibody (BiosPacific Inc., USA)	250.00	1 mg	0.1 μg	0.03
Mouse anti-CA19-9 monoclonal antibody (BiosPacific Inc., USA)	285.00	1 mg	0.1 μg	0.03
Mouse monoclonal antibody against HAS (Santa Cruz Biotechnology, Inc., USA)	307.00	1 mg	0.1 μg	0.03
Goat anti-AFP polyclonal antibody (BiosPacific Inc., USA)	357.00	1 mg	0.12 μg	0.04
Goat anti-CEA polyclonal antibody (BiosPacific Inc., USA)	357.00	1 mg	0.08 μg	0.03
Goat anti-albumin polyclonal antibody (Sigma-Aldrich, USA)	459.00	1 mg	0.09 μg	0.04
HRP-conjugated donkey anti-goat antibody (Jackson ImmunoResearch Laboratories, Inc., USA)	311.00	1 mg	0.12 μg	0.04
HRP-conjugated mouse anti-CA19-9 monoclonal antibody (Wason Biotech Inc., China)	290.00	1 mg	0.04 μg	0.01
HRP-conjugated mouse anti-CA 125 monoclonal antibody (Wason Biotech Inc., China)	257.00	1 mg	0.3 μg	0.08
Chemiluminescent peroxidase substrate (Sigma-Aldrich, USA)	293.00	100 mL	5 μL	0.01
Phosphate buffered saline (PBS) with 0.05% Tween-20 (PBST) (Thermo Fisher Scientific, USA)	110.00	200 tablets 100 mL/tablet	20 μL	<0.01
PDMS microfluidic layer (Home-made)	-	-	1 piece	2.00
OPPolymerSlide™ D glass substrate (CapitalBio Technology, China)	160.00	25 pieces	1 piece	6.40
Disposable tray (Home-made)	-	-	1 piece	0.02
			Total cost:	**8.84**