Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information for

A Microfluidic Plasma Separation Device Combined with Surface Plasmon Resonance for Biomarker Detection in Whole Blood

Table S1 Information about IgG and IgM protein used in our study.

Sample	Host species	Molecular weight (kDa)	Purity	Description	Concentration	Physical form
Native Human IgG Protein (ab91102)	Human	150	95 % SDS-PAGE	Full length protein	10mg (wt)	Lyophilized
Anti-Human IgG antibody [IG266] (ab200699)	Mouse	75	Protein A/G purified	Monoclonal	500 µl at 0.2 mg/ml	Liquid
Native Human IgM Protein (ab91117)	Human	950	95 % SDS-PAGE	Full length protein	1 mg at 1.33 - 1.98 mg/ml	Liquid
Anti-IgM antibody [KT16] (ab110653)	Mouse	N/A	Protein A purified	Monoclonal	100 µg at 1 mg/ml	Liquid

Flow cytometry analysis:

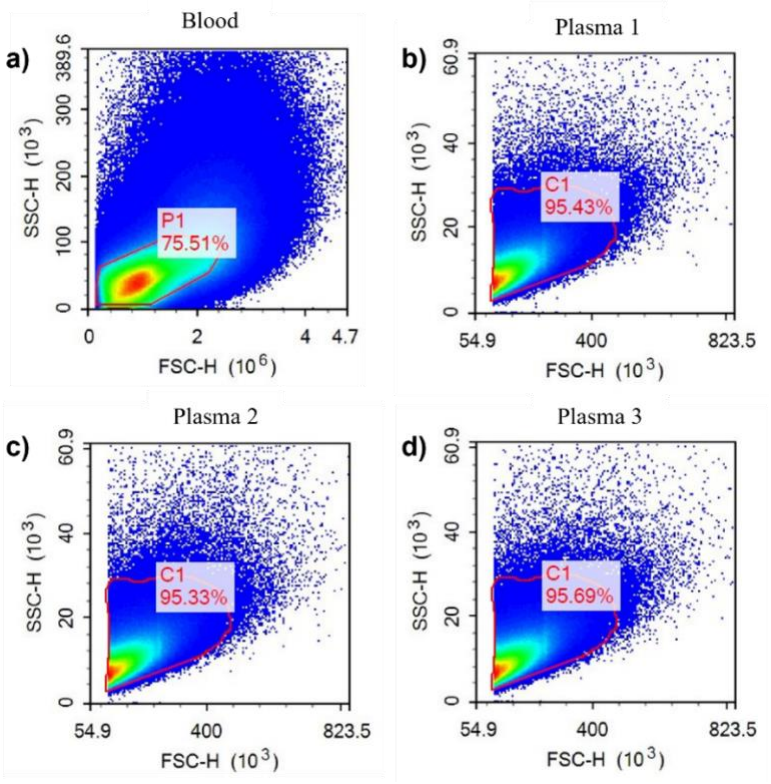

Fig. S1 Flow cytometry analysis of blood samples vs collected plasma before and after filtration using PPS-V2 device. (a) Blood sample and (b), (c), (d) filtered plasma samples with same gating. Blood cells (red) and platelets (blue) are gated on forward scatter (FSC-H) and side scatter (SSC-H).

Flow cytometry analysis was performed for the collected plasma from the PPS-V2 device outlet. At first, 22µl of plasma is collected form the outlet of the three PPS-V2 devices to prepare three plasma samples from the same whole blood sample. All collected plasma samples were diluted (1:10 dilution ratio) in PBS buffer solution containing 1× phosphate-buffered saline (PBS), 0.05% sodium azide (NaN_3) and 2 g/l bovine serum albumin (BSA) to avoid cell adhesion to the channel walls. Four samples of 300 µl each diluted blood and plasma samples were prepared for the flow cytometry analysis and analyzed write after filtration. All cells were counted through flow cytometry (NovoCyte Flow Cytometer, Agilent Technologies, San Diego, CA, USA) and Figure S1 is generated for unfiltered blood and filtered plasma samples. Blood cells were assessed from the dot plot representation and subsequently gated through forward (FSC) and side scatter (SSC) and set to a linear scale (Fig. S1). From Figure S1 (a), the overlap of RBC and WBC populations in forward and side scatter were counted together as blood cells and same gating were applied to plasma samples for comparison.

The purity (*P*) of the PPS-V2 device is calculated using equation 1.s

$$P = \frac{\#of\ filtered\ cell}{\#of\ total\ cell}\% \quad \text{.....(1)}$$

The number of filtered cells were calculated form the flow cytometry data as shown in Table S2.

Table S2: Flow cytometry data for the blood and filtered plasma samples using PPS-V2 device.

Sample	Count	Abs. Count	% Parent	Median X	Median Y	Filtered cell	Purity
Blood	2712630	1176720800	75.51	953543	44518		
Plasma 1	805437	164663600	95.43	150234	9770	1012057200	86.00657012
Plasma 2	764390	156168200	95.33	150863	9690	1020552600	86.72852558
Plasma 3	783474	160118600	95.69	151731	9644	1016602200	86.39281298

Our system recorded a purity of 86.37. The yield (*Y*) of the system is calculated using the formula below (equation 2).

$$Y = \frac{Collected\ volume\ of\ plamsa\ using\ our\ system}{Collected\ volume\ of\ plasma\ for\ ideal\ condition}\% \quad \text{.....(2)}$$

Our system recorded a yield of 25% which is comparable to a high performing whole blood-plasma filtration system.

Table S3. Comparison between PPS (Version 1) and PPS-V2

Performance parameters	PPS (Version 1)	PPS-V2
Collected plasma volume	22ul	22ul
Blood inlet size	80-160ul	80-160ul
Dead-end volume	9ul	4.9ul
Method for separation	Sedimentation and primary separation by membrane	Sedimentation and primary separation by self-built-in filter and secondary separation by membrane
Driving force	Capillary and micropump	Capillary and micropump
Purity	~90%	~86%
Need for parafilm	Yes (to seal inlet for inversion)	No
Need for inversion technique	Yes	No
Filtration time	20 min (for inversion)	15 min
Extraction efficiency (yield)	25%	25%
Ease of integration	Not easy because of inversion technique, leakage through parafilm	Easy to integrate at outlet

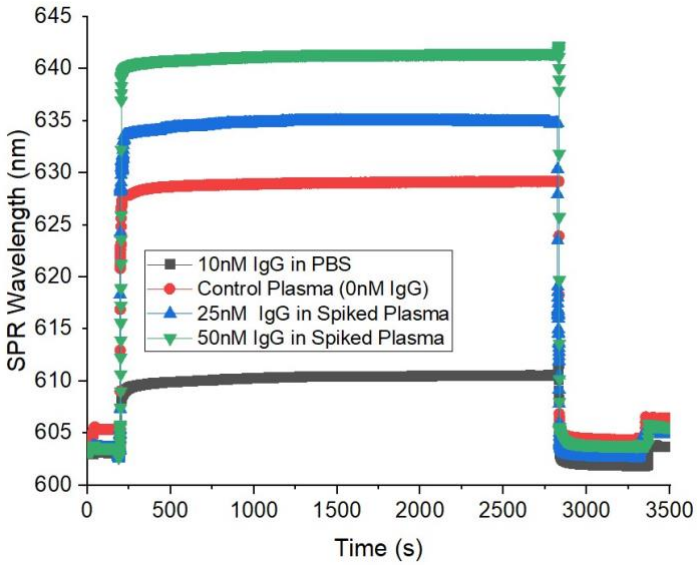

Fig. S2 SPR Wavelength (nm) vs time (s) response for IgG in spiked plasma and PBS.

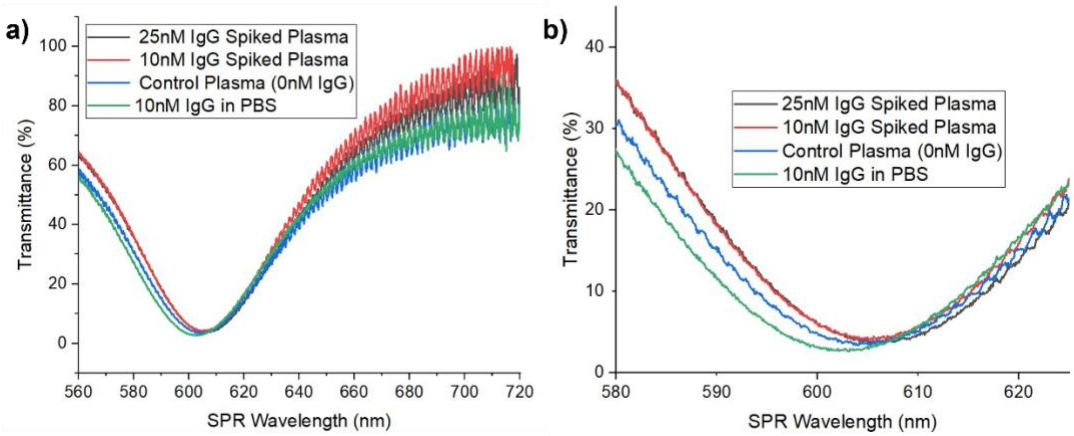

Fig. S3 (a) SPR wavelength vs transmittance for 10nM IgG in PBS, 25nM and 50 nM IgG spiked blood-plasma. (b) The enlarged version of the same showing SPR wavelength shift compared to control plasma.

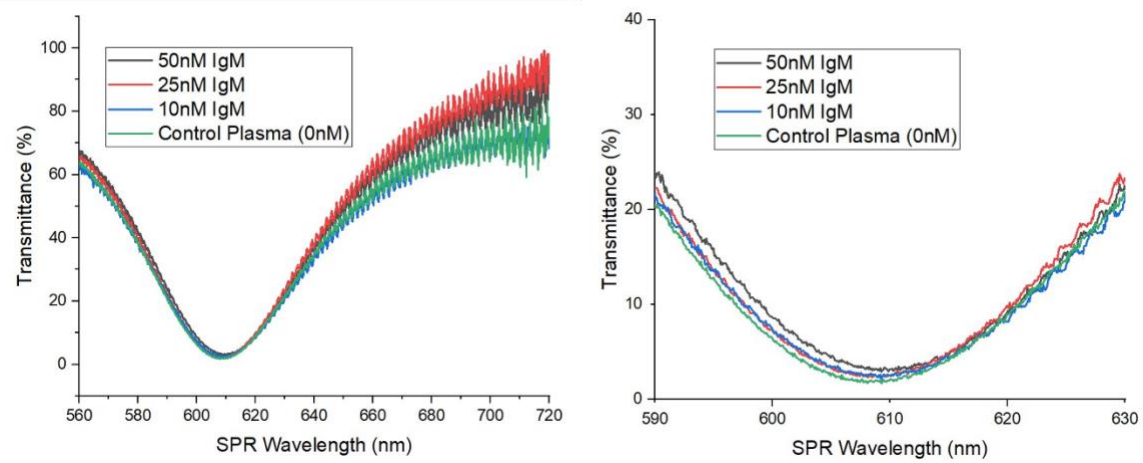

Fig. S4 (a) SPR wavelength vs transmittance for 10, 25 and 50 nM IgM spiked blood-plasma. (b) The enlarged version of the same showing SPR wavelength shift compared to control plasma.