

## Electronic Supporting Information

### **Integrated Strategy for Breast Cancer Biomarker Analysis Using Dual Ionic Liquid Aqueous Biphasic Systems and Microfluidic Immunoassays**

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## ESI Tables

### *IL-IL-ABS for protein depletion and HER2 extraction*

**Table S1.** Recovery yield of IgG ( $RY_{IgG}$ , %) and HSA ( $RY_{HSA}$ , %) in the top and bottom phases and depletion efficiencies of IgG ( $DE_{IgG}$ , %) and HSA ( $DE_{HSA}$ , %) achieved with ABS containing 30 or 40 wt% phosphonium-based IL or PPG 400, 35 wt% cholinium-based IL or  $K_3C_6H_5O_7/C_6H_8O_7$ , 33 or 23 wt% PBS and 2 wt% of human serum.

ABS	IgG			HSA		
	$RY_{Top}$ (%)	$DE_{IgG}$ (%)	$RY_{Bot}$ (%)	$RY_{Top}$ (%)	$DE_{HSA}$ (%)	$RY_{Bot}$ (%)
$[P_{4442}][Et_2PO_4] + [Ch][H_2PO_4]$	$33 \pm 5$	$67 \pm 5$	$0.7 \pm 0.3$	$70 \pm 6$	$30 \pm 6$	0
$[P_{4444}]Cl + [Ch][H_2PO_4]$	$14 \pm 6$	$86 \pm 6$	0	$63 \pm 6$	$37 \pm 6$	0
$[P_{4441}][CH_3SO_4] + [Ch][H_2PO_4]$	$4 \pm 1$	$91 \pm 2$	$6 \pm 1$	$25 \pm 4$	$75 \pm 4$	0
$[P_{i(444)1}][TsO] + [Ch][H_2PO_4]$	0	100	0	$31 \pm 6$	$69 \pm 6$	0
$[P_{4444}]Br + [Ch][H_2PO_4]$	0	100	0	0	100	0
$[P_{4444}]Br + [Ch][CH_3COO]$	0	100	0	0	100	0
PPG 400 + $[Ch][H_2PO_4]$	$20 \pm 2$	$3 \pm 3$	$78 \pm 2$	$22 \pm 6$	$78 \pm 6$	0
$[P_{i(444)1}][TsO] + K_3C_6H_5O_7/C_6H_8O_7$	0	100	0	$55 \pm 1$	$45 \pm 1$	0
PPG 400 + $K_3C_6H_5O_7/C_6H_8O_7$	$18 \pm 3$	$80 \pm 2$	$2 \pm 1$	$17 \pm 1$	$83 \pm 1$	0

**Table S2.** Recovery yields of HER2 ( $RY_{HER2}$ , %) in the top phase of ABS containing 30 wt% phosphonium-based IL or PPG 400, 35 wt% cholinium-based IL or  $K_3C_6H_5O_7/C_6H_8O_7$ , 33 wt% PBS and 2 wt% HER2 aqueous solution ( $15 \text{ ng} \cdot \text{mL}^{-1}$ ).

ABS	$RY_{HER2}$ (%)
$[P_{4441}][CH_3SO_4] + [Ch][H_2PO_4]$	$95 \pm 3$
$[P_{i(444)1}][TsO] + [Ch][H_2PO_4]$	$102 \pm 8$
$[P_{4444}]Br + [Ch][H_2PO_4]$	0
$[P_{4444}]Br + [Ch][CH_3COO]$	0
PPG 400 + $[Ch][H_2PO_4]$	$100 \pm 7$
$[P_{i(444)1}][TsO] + K_3C_6H_5O_7/C_6H_8O_7$	$112 \pm 7$
PPG 400 + $K_3C_6H_5O_7/C_6H_8O_7$	$97 \pm 4$

**Table S3.** Recovery yields of HER2 ( $RY_{HER2}$ , %) in the top, inter and bottom phases of ABS containing 30 wt% phosphonium-based IL or PPG 400, 35 wt% cholinium-based IL or  $K_3C_6H_5O_7/C_6H_8O_7$ , 33 wt% PBS and 2 wt% HER2 spiked human serum ( $15 \text{ ng}\cdot\text{mL}^{-1}$ ).

ABS	$RY_{HER2}$ (%)		
	Top phase	Interphase	Bottom phase
$[P_{4441}][CH_3SO_4] + [Ch][H_2PO_4]$	$52 \pm 5$	$49 \pm 5$	0
$[P_{i(444)1}][TsO] + [Ch][H_2PO_4]$	$93 \pm 2$	$7 \pm 2$	0
PPG 400 + $[Ch][H_2PO_4]$	$66 \pm 4$	$34 \pm 4$	0
$[P_{i(444)1}][TsO] + K_3C_6H_5O_7/C_6H_8O_7$	$62 \pm 3$	$38 \pm 3$	0
PPG 400 + $K_3C_6H_5O_7/C_6H_8O_7$	$52 \pm 2$	$48 \pm 2$	0

**Table S4.** Depletion efficiencies of IgG ( $DE_{IgG}$ , %) and HSA ( $DE_{HSA}$ , %) and recovery yields of HER2 ( $RY_{HER2}$ , %) in the top, inter and bottom phases of ABS 30 wt%  $[P_{i(444)1}][TsO] + 35 \text{ wt}\%$   $[Ch][H_2PO_4] + 33/30/25 \text{ wt}\%$  PBS + 2/5/10 wt% human serum.

Serum composition (wt%)	DE (%)		$RY_{HER2}$ (%)		
	IgG	HSA	Top phase	Interphase	Bottom phase
2	100	$69 \pm 6$	$93 \pm 2$	$7 \pm 2$	0
5	100	$74 \pm 2$	$94 \pm 2$	$6 \pm 2$	0
10	100	$84 \pm 1$	$97 \pm 3$	$3 \pm 3$	0

### HER2 detection in microfluidic devices

**Table S5.** Fluorescence intensity measurements in various sample matrices for HER2 concentrations from 0 to  $25 \text{ ng}\cdot\text{mL}^{-1}$  in PBS, untreated serum, ABS top phase (without serum), and ABS top phase after serum pretreatment. Data used for sensitivity curves, LoD and RSD (%) determination. Signal ratios between the highest HER2 concentration ( $25 \text{ ng}\cdot\text{mL}^{-1}$ ) and the negative control ( $0 \text{ ng}\cdot\text{mL}^{-1}$ ) are also included and highlighted in grey to facilitate the comparison of fold increases across different matrices.

[HER2] ( $\text{ng}\cdot\text{mL}^{-1}$ )	Fluorescence Intensity (A.U.)			
	PBS	Human serum	ABS top phase	ABS top after serum pretreatment
0	$12375 \pm 376$	$10694 \pm 205$	$7517 \pm 607$	$6260 \pm 390$
5	$12585 \pm 478$	$10609 \pm 1302$	$7358 \pm 167$	$6101 \pm 310$
10	$13262 \pm 1292$	$11770 \pm 1027$	$8551 \pm 323$	$6198 \pm 612$
15	$14563 \pm 815$	$12207 \pm 969$	$11148 \pm 912$	$7466 \pm 647$
20	$20539 \pm 1493$	$13601 \pm 495$	$14561 \pm 273$	$8263 \pm 555$
25	$25089 \pm 1693$	$15072 \pm 347$	$15935 \pm 691$	$9231 \pm 574$
Signal ratio	2.03	1.41	2.12	1.47