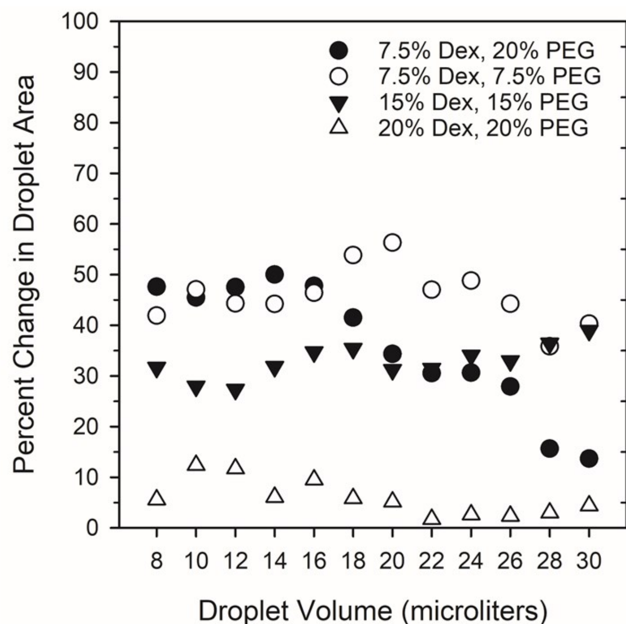
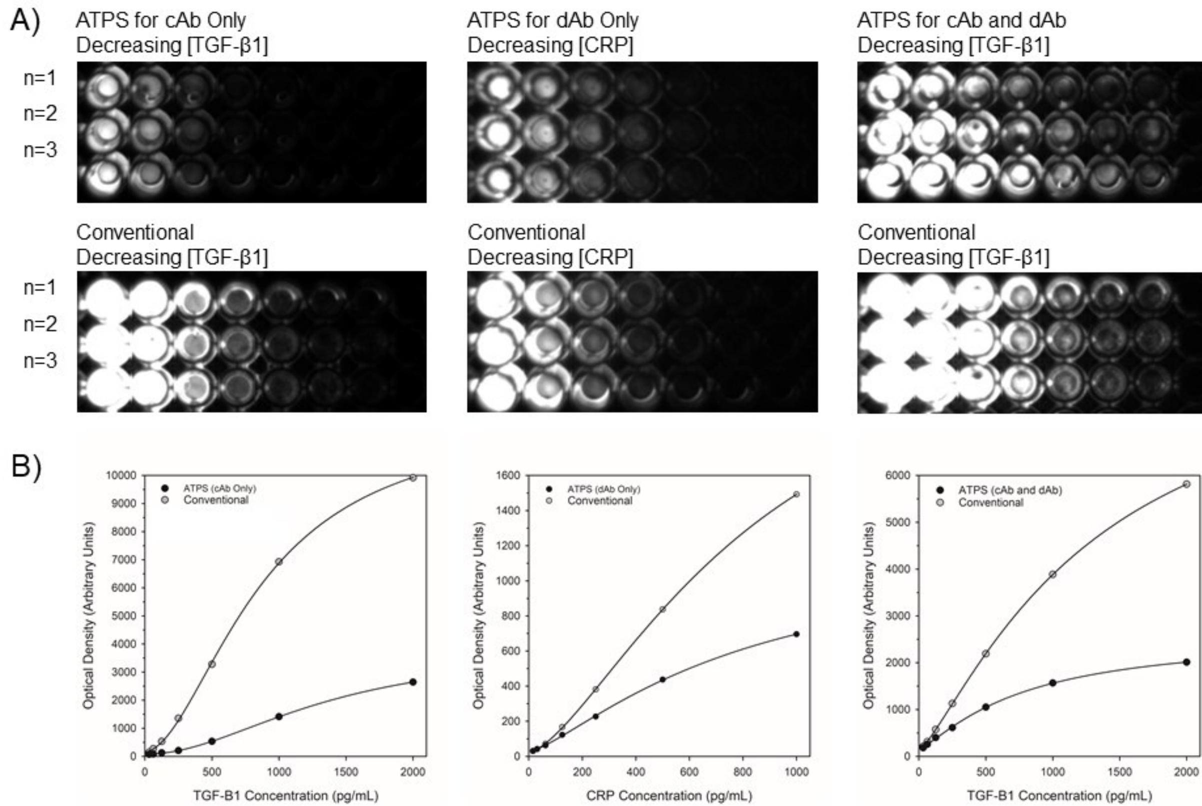
Electronic Supplementary Material (ESI) for Analyst.
This journal is © The Royal Society of Chemistry 2020

ELECTRONIC SUPPLEMENTARY INFORMATION

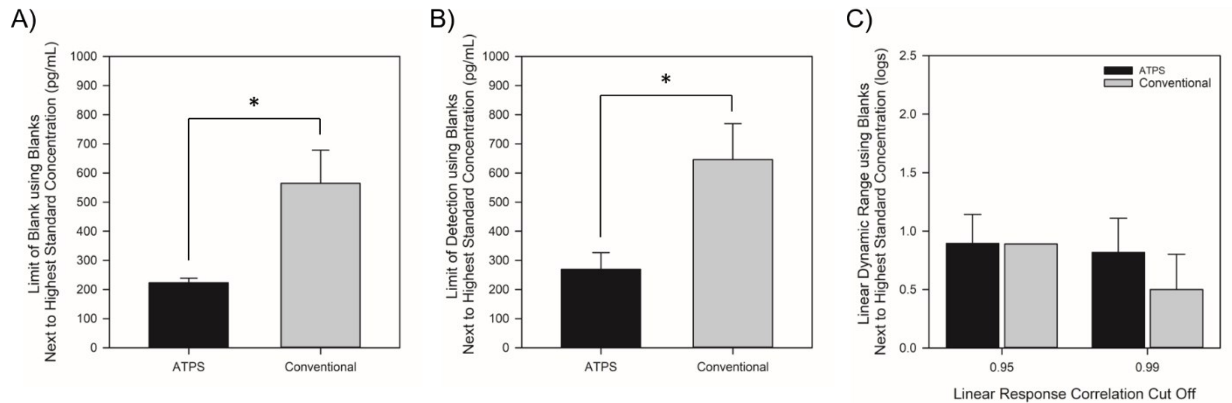


Supplemental Figure 1. Optimization of polymer concentrations. Dextran droplet volumes for various ATPS formulations were varied and the percentage change in area of the dextran droplets after 24 hours was recorded. Area coverage decreased for a fixed volume as polymer concentration increased due to the larger liquid-liquid-plate interfacial tension present. For equal concentrations of polymers, droplet area remained consistent over time. However, for the 7.5% dextran, 20% PEG system, the droplet gradually shrunk due to equilibration of the two polymer solutions.

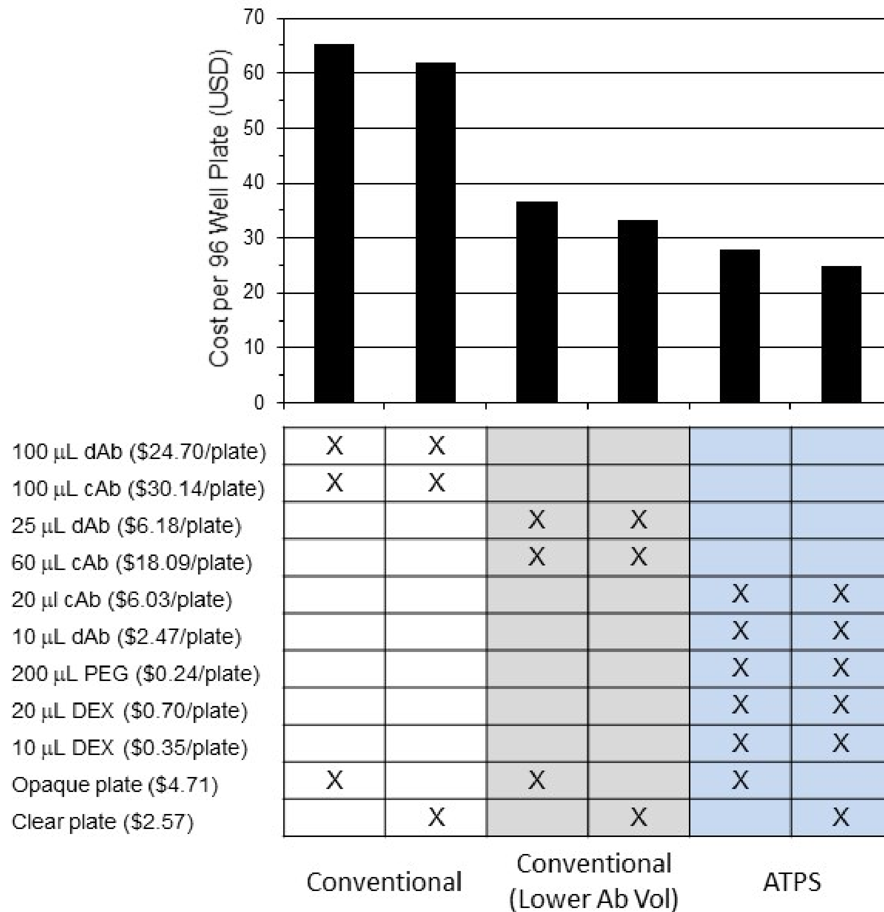


Supplemental Figure 2. A) Representative images for assays in which an ATPS is used to dispense just the capture antibody (cAb), just the detection antibody (dAb) or both the capture and detection antibodies. Images of the conventional ELISA performed within the same plate are shown for comparison. For the trials using only ATPS for the cAb step, the TGF-β1 ELISA kit was used. The capture and detection antibody concentrations used for the TGF-β1 kit were 2 μg/mL and 300 ng/mL, respectively. The volumes used for the capture antibody in the ATPS condition was 20 μL whereas, the volume of the capture antibody for the conventional condition was 60 μL. The detection antibody volume for both ATPS and conventional conditions was 50 μL. For the trials using only ATPS for the dAb step, the CRP ELISA kit was used. The capture and detection antibody concentrations used for the CRP kit were 2 μg/mL and 90 ng/mL, respectively. The volume of capture antibody used for both the conventional and ATPS conditions was 50 μL. The volume of detection antibody used for the conventional condition was 25 μL whereas, the volume of detection antibody used for the ATPS condition was 10 μL. For the trials using ATPS for both the cAb and dAb step, the TGF-β1 kit was used. The capture and detection antibody concentrations used for the TGF-β1 kit were 2 μg/mL and 300 ng/mL,

respectively. The volumes used for the capture and detection antibody solutions for the ATPS conditions were 20 μL and 10 μL, respectively. The volumes used for the capture and detection antibody solutions for the conventional conditions were 60 μL and 25 μL, respectively. **B)** Representative standard curves comparing signal versus concentration between APTS-ELISA and conventional sandwich ELISA.



Supplemental Figure 3. Performance characteristics of ATPS-ELISA compared to conventional sandwich ELISA. The limit of blank **(A)** and limit of detection **(B)** were calculated using blank wells next to the highest standard concentration of TGF-β1 (2000 pg/mL) for both ATPS-ELISA (using capture and detection antibody in ATPS) and conventional sandwich ELISA. **C)** Average linear dynamic ranges of TGF-β1 standard curves for ATPS-ELISA and conventional sandwich ELISA determined using different correlation coefficients as maximum linear response cut offs using the integrated densities of wells adjacent to the highest standard concentration of TGF-β1 (2000 pg/mL). For the ATPS conditions, both the capture and detection antibodies were dispensed in dextran. Capture and detection antibody concentrations used were 2μg/mL and 300 ng/mL, respectively. The volumes used for the capture and detection antibody solutions for the ATPS conditions were 20 μL and 10 μL, respectively. The volumes used for the capture and detection antibody solutions for the conventional conditions were 60 μL and 25 μL, respectively. Error bars represent standard deviation of the mean. The * indicates $p<0.05$.



	Conventional		Conventional (Lower Ab Vol)		ATPS	
100 µL dAb ($24.70/plate)	X	X				
100 µL cAb ($30.14/plate)	X	X				
25 µL dAb ($6.18/plate)			X	X		
60 µL cAb ($18.09/plate)			X	X		
20 µl cAb ($6.03/plate)					X	X
10 µL dAb ($2.47/plate)					X	X
200 µL PEG ($0.24/plate)					X	X
20 µL DEX ($0.70/plate)					X	X
10 µL DEX ($0.35/plate)					X	X
Opaque plate ($4.71)	X		X		X	
Clear plate ($2.57)		X		X		X

Supplemental Figure 4. Comparison of assay costs. Each bar in the graph (x-axis) corresponds to a column in the table and represents the costs (shown on the left) by varying the volumes of capture/detection antibodies, with/without ATPS polymers, and using different type of plates.