Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

One-Incubation One-Hour Multiplex ELISA Enabled by Aqueous Two-Phase Systems

Mintra Tongdee, [a,b] Cameron Yamanishi,[b] Midori Maeda,[b] Taisuke Kojima,[b] John Dishinger,[c] Rattikan Chantiwas[a] and Shuichi Takayama[b*]

[a] Department of Chemistry and Center of Excellence for Innovation in Chemistry and Flow Innovation-Research for Science and Technology Laboratories (FIRST Labs), Faculty of Science, Mahidol University, Rama VI Rd., Bangkok 10400, Thailand

[b] Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta 30332, Georgia, USA

[c] PHASIQ, Inc., Ann Arbor, Michigan 48109, USA

* Corresponding author: takayama@gatech.edu

Content

1. Optimization of one-incubation one-hour ATPS ELISA

1.1 PEG-DEX concentration

1.2 PHASIQ plate

1.3 Incubation time

1.4 Blocking buffer

2. Previous reports of ELISA for cytokine detection

3. Companion image processing software as an ImageJ plugin

ESI 1. Optimization of one-incubation one-hour ATPS ELISA

1.1 PEG-DEX concentration

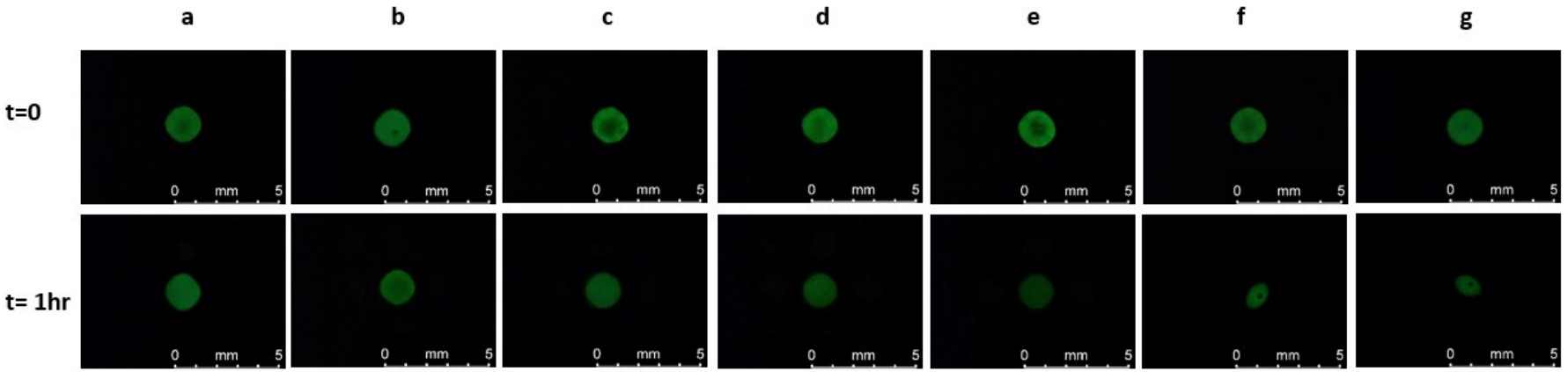


Fig. S1 Effect of PEG-DEX concentration on FITC-dAb retention in DEX over the course of 1 hour at room temperature. PEG-DEX concentration during the assay (%w/w); (a) 9%PEG-0.81%DEX, (b) 5%PEG-0.81%DEX, (c) 9%PEG-0.45%DEX, (d) 5%PEG-0.45%DEX, (e) 5%PEG-0.27%DEX, (f) 3%PEG-0.45%DEX and (g) 3%PEG-0.27%DEX.

See Fig. S2 (Section 1.2) for picture of PHASIQ plate used for this study.

1.2 PHASIQ plate

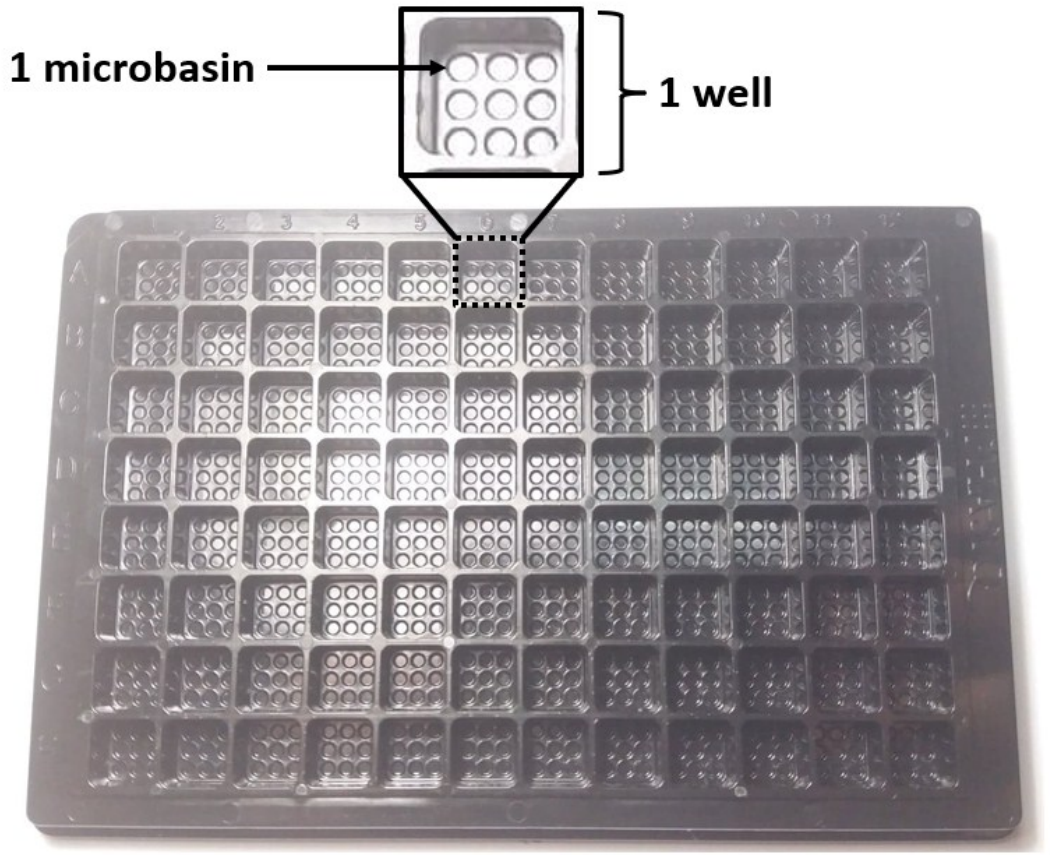


Fig. S2 Picture of PHASIQ plate with zoomed in picture. A single well contained nine microbasins.

1.3 Incubation time

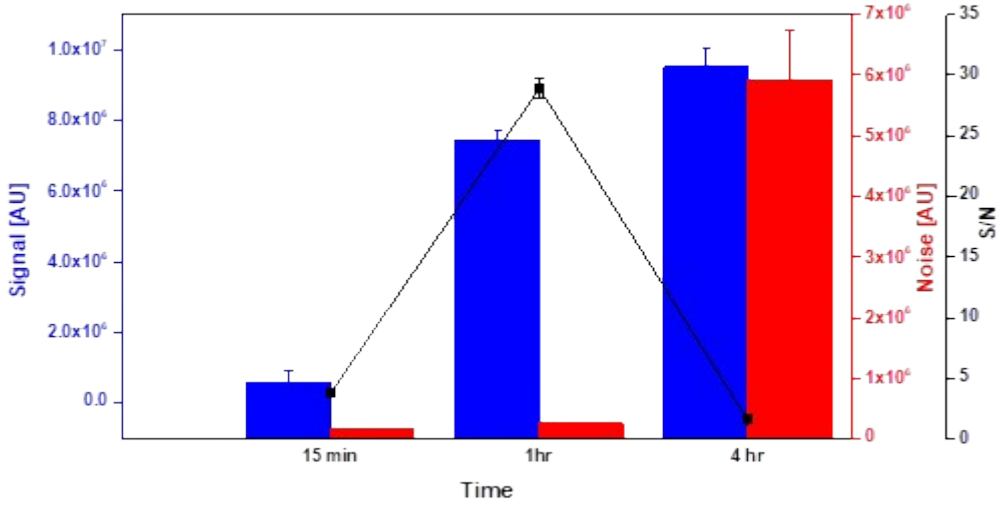


Fig. S3 Signal, noise and ratio of S/N for incubation time of 15 minutes, 1 hour and 4 hours.

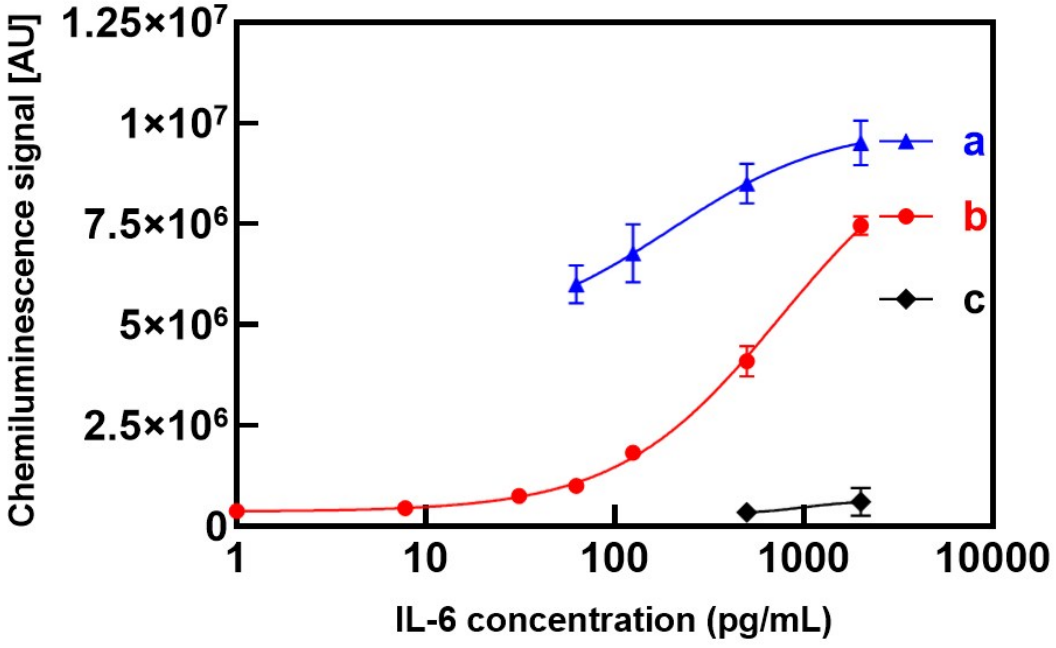


Fig. S4 Calibration data for analysis of IL-6 with one-incubation ATPS ELISA using different incubation time; (a) 4 hours, (b) 1 hour and (c) 15 minutes and the calculated LODs were 180, ~1 and 340 pg mL^{-1}, respectively. Data shown are mean chemiluminescence signals from three replicates, and error bars are standard deviations (SDs).

1.4 Blocking buffer

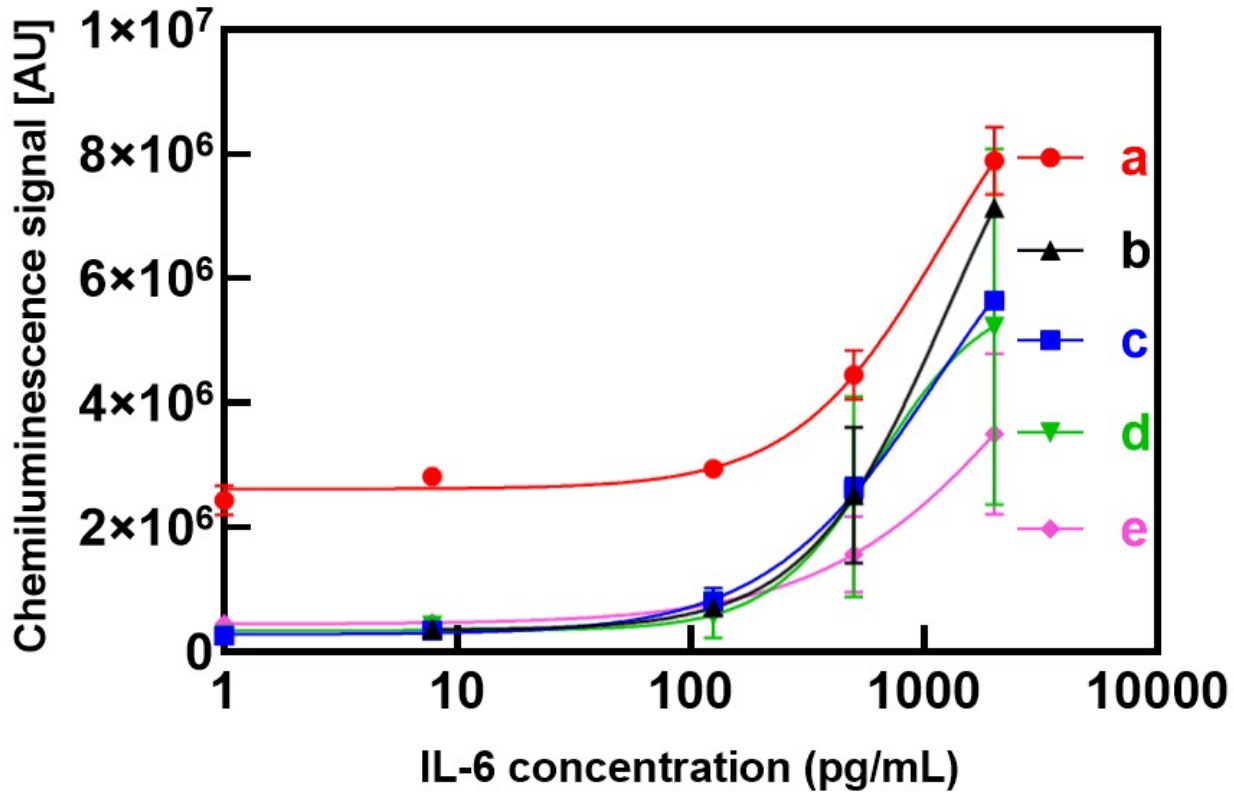


Fig. S5 Calibration data for analysis of IL-6 with one-incubation ATPS ELISA using different types of blocking buffer; (a) 0.1%Chonblock/ 0.05%goat serum, (b) 1×StabilCoat, (c) 3×StabilCoat, (d) 5%BSA and (e) 5%goat serum and the calculated LODs were 100, 20, ~1, 100 and 60 pg mL^{-1}, respectively. Data shown are mean chemiluminescence signals from three replicates, and error bars are standard deviations (SDs).

ESI 2. Previous reports of ELISA for cytokine detection

Table S1 Literature review of various detection techniques for multiplex cytokine detection, assay information included; types of cytokines, sample volume, assay time and LOD.

Detection technique	Types of cytokines	Sample volume	Assay time	LOD (pg mL^{-1})	Ref.
FI	IL-6, IFN-γ	20 μL	2 hours	7.8	Wang and Zhang 2006[1]
FI	VEGF, EGF, IP10, IL-8, MCP-1, IL-6, TIMP-1, MIP-1β, RANTES, Eotaxin-2	100 μL	2.5 hours	0.01-8	Blicharz et. al. 2009[2]
FI	IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α	50 μL	3 hours	40.96	Hall et. al. 2015[3]
EC	VEGF, IL-8, TIMP-1	N/A	2.5 hours	N/A	Deiss et. al. 2009[4]
EC	TNF-α, IFN-γ, IL-2	4 μL	1 hours	N/A	Stybayeva et. al. 2010[5]
EC	IL-1α, L-1β, L-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN-γ, EGF, MCP-1, TNF-α	100 μL	2 hours	0.12-2.12	FitzGerald et. al. 2007[6]
SPR	IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ	1 μL	40 minutes	5-20	Chen et. al. 2015[7]

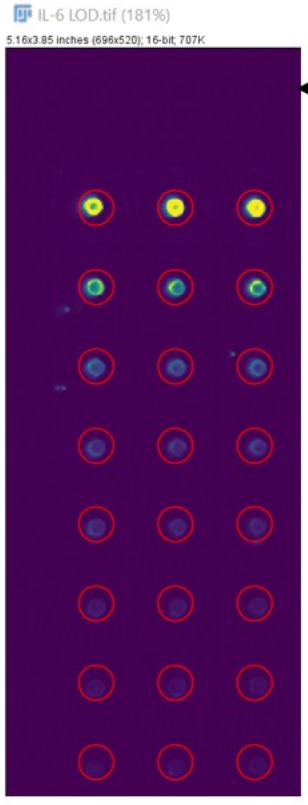
Detection technique	Types of cytokines	Sample volume	Assay time	LOD (pg mL^{-1})	Ref.
SPR	IL-1β, L-6, TNF-α	N/A	10 minutes	200-1,300	Battaglia et. al. 2005[8]
MR	IL-2, IL-6, IL-8	N/A	90 minutes	≤ 1	Kindt et. al. 2013[9]
Spectrocolorimeter	IL-6, IL-8	N/A	45 minutes	100	Miwa et. al. 2009[10]
AIR	IL-1α, L-1β, L-6, IL-8, IL-10, IFN-γ, TNF-α	N/A	N/A	< 10	Carter et. al. 20[11]
Bioluminescence (bioassay)	IL-6, IL-8, THF-α	N/A	N/A	37-184	Yu et. al. 2019[12]
Imager of the automated integrated microfluidic device	VEGF, IP-10, IL-8, EGF, MMP-9, IL-1β	10 μL	70 minutes	4-8,624	Nie et. al. 2014[13]
Fluorescent Bead-Based Luminex Cytokine Assays	IFN-γ, TNF-α, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-12-p70, IL-13, IL-17, GM-CSF, MCP-1	25–50 μl	2-3 hours	N.A.	Joel et. al. 2008[14]
Quanterix SIMOA instrumentation	IL-6, TNF-α, IL-1β, IL-8	100 μL	45 minutes	N.A.	Joachim et. al. 2015[15]

Detection technique	Types of cytokines	Sample volume	Assay time	LOD (pg mL^{-1})	Ref.
Aushon SearchLight Protein Array Technology (Chemiluminescent label)	IL-1α, IL-1β, IL-6, TNF-α, VEGF, IL-8/MIP-2	100 μL	1-2 hours	N.A.	Dennis et. al. 2010[16]
Chemiluminescence (ELISA Microarray)	IL-1β, IL-1ra, IL-6, IL-8, MCP-1, TNF-α	50 μL	4.5 hours	15-320	Urbanowska et. al. 2006[17]
Chemiluminescence (ELISA)	IL-6, IL-10, TNF-α, IL-1β, CCL18	100 μL	1 hour	1.82-7.63	This work

FI: Fluorescence immunoassay
EC: Electrochemical method
SPR: Surface plasmon resonance
MR: Microring resonator based immunosensing
AIR: Arrayed imaging reflectometry
N/A: Not available

ESI 3. Companion image processing software as a Fiji image J plugin

This plugin deconvolves an image of a PHASIQ-layout plate. Software details of custom Fiji image J plugin is given below.



1. The plugin guides the user through image rotation and determination of the size and locations of microbasins to generate a plate-wide map.

PHASIQ Analyzer Results

Export to Excel | Open File on Export

Column	Row	Microbasin Label	Intensity	StdDev
1	A	5	7145358.0	24242.32674327188
1	B	5	4574092.0	16532.716025658694
1	C	5	2013680.0	7023.245983372411
1	D	5	1201348.0	3952.2273081058556
1	E	5	982464.0	3167.829983122191
1	F	5	539804.0	1612.537882624684
1	G	5	414804.0	1233.0405314613217
1	H	5	309116.0	905.4306069584926
2	A	5	7724410.0	25438.68091252201
2	B	5	3634959.0	13921.362625331285
2	C	5	1713016.0	6068.928171622032
2	D	5	901064.0	3161.2071509126654
2	E	5	689520.0	2329.3406343710385
2	F	5	495560.0	1536.4198284676006
2	G	5	430012.0	1211.0096145005057
2	H	5	296184.0	919.3759071813021

2. The plugin then measured the average chemiluminescence intensity for each microbasin, exporting an Excel sheet with annotated microwells and microbasin intensities.

References

1. C. Wang and Y. Zhang, *Sens. Actuators, B*, 2006, **120**, 125-129.
2. T. M. Blicharz, W. L. Siqueira, E. J. Helmerhorst, F. G. Oppenheim, P. J. Wexler, F. F. Little and D. R. Walt, *Anal. Chem.*, 2009, **81**, 2106-2114.
3. S. A. Hall, D. Stucke, B. Morrone, D. Lebelt and A. J. Zanella, *MethodsX*, 2015, **2**, 241-248.
4. F. Deiss, C. N. LaFratta, M. Symer, T. M. Blicharz, N. Sojic and D. R. Walt, *J. Am. Chem. Soc.*, 2009, **131**, 6088-6089.
5. G. Stybayeva, O. Mudanyali, S. Seo, J. Silangcruz, M. Macal, E. Ramanculov, S. Dandekar, A. Erlinger, A. Ozcan and A. Revzin, *Anal. Chem.*, 2010, **82**, 3736-3744.
6. S. P. FitzGerald, R. I. McConnell and A. Huxley, *J. Proteome Res.*, 2008, **7**, 450-455.
7. P. Chen, M. T. Chung, W. McHugh, R. Nidetz, Y. Li, J. Fu, T. T. Cornell, T. P. Shanley and K. Kurabayashi, *ACS Nano*, 2015, **9**, 4173-4181.
8. T. M. Battaglia, J.-F. Masson, M. R. Sierks, S. P. Beaudoin, J. Rogers, K. N. Foster, G. A. Holloway and K. S. Booksh, *Anal. Chem.*, 2005, **77**, 7016-7023.
9. J. T. Kindt, M. S. Luchansky, A. J. Qavi, S.-H. Lee and R. C. Bailey, *Anal. Chem.*, 2013, **85**, 10653-10657.
10. K. Miwa, N. Shibayama, T. Moriguchi, J. Goto, M. Yanagisawa, Y. Yamazaki, G. Jung and K. Matsuda, *J. Clin. Lab. Anal.*, 2009, **23**, 40-44.
11. J. A. Carter, S. D. Mehta, M. V. Mungillo, C. C. Striemer and B. L. Miller, *Biosens. Bioelectron.*, 2011, **26**, 3944-3948.
12. X. Yu, D. Scott, E. Dikici, S. Joel, S. Deo and S. Daunert, *Analyst*, 2019, **144**, 3250-3259.
13. S. Nie, W. H. Henley, S. E. Miller, H. Zhang, K. M. Mayer, P. J. Dennis, E. A. Oblath, J. P. Alarie, Y. Wu and F. G. Oppenheim, *Lab on a Chip*, 2014, **14**, 1087-1098.
14. J. F. Djoba Siawaya, T. Roberts, C. Babb, G. Black, H. J. Golakai, K. Stanley, N. B. Bapela, E. Hoal, S. Parida, P. van Helden and G. Walzl, *PLoS One*, 2008, **3**, e2535.
15. J. D. Pleil, M. M. Angrish and M. C. Madden, *J. Breath Res.*, 2015, **9**, 047108.
16. D. R. Trune, B. E. Larrain, F. A. Hausman, J. B. Kempton and C. J. MacArthur, *Hear. Res.*, 2011, **275**, 1-7.
17. T. Urbanowska, S. Mangialaio, C. Zickler, S. Cheevapruk, P. Hasler, S. Regenass and F. Legay, *J. Immunol. Methods*, 2006, **316**, 1-7.