

Profiling Cytokine Levels Using A Multiplex Human Cytokine ELISA Array

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Abstract

The induction of multiple cytokines is a common phenomenon in many immunological responses; therefore, a method to quickly and simultaneously screen *in vitro* or *in vivo* samples for multiple cytokine levels is needed. We developed a "multiplex" sandwich ELISA Array on a 96-well plate allowing researchers to screen several human serum, plasma or cell culture supernatant samples for multiple cytokines in one experiment. The ELISA Array is easy to perform and only requires a standard ELISA plate reader. It can be also used in conjunction with corresponding single analyte ELISA kits to perform validation and absolute quantification. As an illustrative example, we monitored the time-dependent (0, 6, 18, 24, and 48 hours) patterns of Th1/Th2 cytokine induction by human peripheral blood mononuclear cells (PBMC) in response to PMA (50 ng/ml) and ionomycin (1 µg/ml). Large amounts of IL-2, IFN-gamma and TNF-alpha are induced after the 6-h stimulation when only more moderate amounts of IL-4, IL-5, IL-10 and IL-13 are secreted. The amount of IL-12 p70 is not detectable even after 48 hours. The ELISA Array thus provides a powerful, easy-to-use solution for profiling the relative levels of several cytokines across multiple experimental conditions at the same time.

Materials & Methods

Multi-Analyte Profiler ELISArray™:

MEH-001A (SuperArray Inc.) for Human Th1 / Th2 Cytokines

Plate Layout:

		1	2	3	4	5	6	7	8	9	10	11	12
IL-2	A	Analyte 1											
IL-4	B	Analyte 2											
IL-5	C	Analyte 3											
IL-10	D	Analyte 4											
IL-12	E	Analyte 5											
IL-13	F	Analyte 6											
IFN _γ	G	Analyte 7											
TNF _α	H	Analyte 8											

Experiments:

Peripheral blood mononuclear cells (PBMC) purchased from AllCells were cultured in DMEM medium with 10% FBS, 1X MEM NEAA, 1X GlutaMax™-1, and 10 mM HEPES. PBMC were stimulated with 50 ng/ml PMA and 1 µg/ml ionomycin. Cell supernatants were collected at different time points (0, 6, 18, 24, and 48 hours) and the cytokine productions were measured by enzyme-linked immunosorbent assay (ELISA) using the Human Th1 / Th2 Cytokines Multi-Analyte Profiler ELISArray™ Kit.

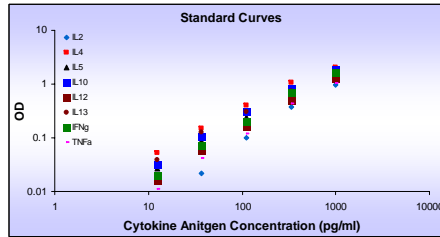
Calculations:

In the standard curve protocol, the readings for each protein analyte from the samples are compared to a standard curve for quantification of the amount of protein in the original samples. In the relative profiling experiment, a non-linear regression method is used to convert absorbance readings into relative changes in the protein levels between samples.

Results: Standard Curve Protocol

Layout	1		2		3		4		5		6		7		8		9		10		11		12	
	Sample 1		Sample 2		Antigen Standard Controls		BKG																	
A	1x sample		1x sample		1x sample		1x sample		1x sample		2000 pg/ml		666 pg/ml		222 pg/ml		74 pg/ml		25 pg/ml		Blank			
B																								
C																								
D																								
E																								
F																								
G																								
H																								

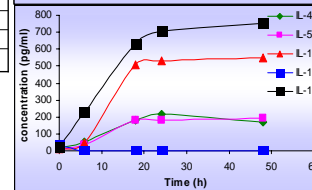
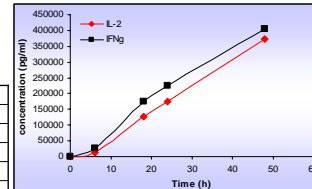
Data	1		2		3		4		5		6		7		8		9		10		11		12	
	Sample 1 (6h)		Sample 2 (18h)		Antigen Standard Controls		BKG																	
IL-2	2.34	0.29	0.080	3.42	1.55	0.28	1.03	0.43	0.16	0.08	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	
IL-4	0.10	0.04	0.04	0.24	0.04	0.04	2.05	1.11	0.44	0.19	0.09	0.09	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	
IL-5	0.08	0.04	0.04	0.19	0.05	0.05	1.62	0.73	0.29	0.14	0.08	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	
IL-10	0.09	0.04	0.04	0.50	0.06	0.04	1.89	0.87	0.35	0.16	0.08	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	
IL-12	0.06	0.06	0.06	0.06	0.06	0.04	1.31	0.56	0.22	0.12	0.08	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	
IL-13	0.20	0.04	0.04	0.46	0.06	0.04	1.32	0.78	0.35	0.17	0.08	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	
IFN _γ	3.95	0.86	0.13	3.94	3.22	0.61	1.69	0.73	0.25	0.12	0.07	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	
TNF _α	1.10	0.09	0.05	2.37	0.14	0.05	1.08	0.48	0.17	0.10	0.07	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	



Cytokine	Linear Fit (R ²)
IL-2	0.997
IL-4	0.944
IL-5	0.986
IL-10	0.980
IL-12	0.994
IL-13	0.906
IFN _γ	0.992
TNF _α	0.992

Summary (unit pg/ml)

	Rest	6 h	18 h	24 h	48 h
IL-2	0.0	12917	126500	173900	373550
IL-4	19.4	50.7	182.1	214.3	170.1
IL-5	13.7	33.6	183.5	183.4	190.8
IL-10	10.4	44.9	506.1	533.2	550.9
IL-12	32.3	N/A	N/A	N/A	N/A
IL-13	21.2	229.5	630.9	707.9	753.1
IFN _γ	0.5	25300	173500	224912	404176
TNF _α	38.3	1819	5521	8170	14475



Results: Relative Profiling Protocol

Layout	1		2		3		4		5		6		7		8		9		10		11		12	
	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		POS		BK		G									
A	1X Sample		100X Sample		1X Sample		100X Sample		1X Sample		100X Sample		1X Sample		100X Sample		1X Sample		100X Sample		20,000 pg/ml		0 pg/ml	
B																								
C																								
D																								
E																								
F																								
G																								
H																								

Four-parameter Logistic-log Model

$$OD = bottom + \frac{Top - bottom}{1 + 10^{(LogEC_{50} - X) \times Hillslope}}$$

$$X = LogEC_{50} - \frac{Log\left(\frac{Top - bottom}{OD - bottom} - 1\right)}{Hillslope}$$

$$X_2 - X_1 = \left(Log\left(\frac{top - bottom}{OD_1 - bottom} - 1\right) - Log\left(\frac{top - bottom}{OD_2 - bottom} - 1\right) \right) / Hillslope$$

$$C_2 / C_1 = 10^{(X_2 - X_1)} \times (Dilution_factor_2) \div (Dilution_factor_1)$$

	1	2	3	4	5	6	7	8	9	10	11	12	Hill Slope	Log EC ₅₀
IL-2	3.67	1.92	2.00	0.11	0.73	0.08	0.35	0.08	0.14	0.07	3.39	0.07	1.14	2.95
IL-4	3.46	3.36	3.38	0.45	3.09	0.13	2.43	0.07	1.06	0.03	3.42	0.02	1.32	1.94
IL-5	3.60	3.31	3.32	0.15	2.22	0.06	1.13	0.04	0.34	0.03	3.53	0.03	1.22	2.82
IL-10	3.71	3.46	3.45	0.18	2.47	0.06	1.30	0.05	0.39	0.02	3.64	0.03	1.19	2.56
IL-12	3.85	2.91	2.94	0.09	1.30	0.04	0.59	0.03	0.18	0.03	3.72	0.03	1.22	2.76
IL-13	3.66	3.49	3.46	0.32	2.93	0.10	1.98	0.05	0.72	0.03	3.60	0.03	1.06	2.36
IFN _γ	3.60	3.32	3.31	0.20	2.62	0.07	1.57	0.04	0.49	0.03	3.51	0.02	1.31	2.29
TNF _α	3.85	3.16	3.21	0.17	1.95	0.10	0.99	0.09	0.31	0.08	3.66	0.07	1.23	2.76

Results		S1/S2	S1/S3	S1/S4	S1/S5	S2/S3	S2/S4	S2/S5	S3/S4	S3/S5	S4/S5
IL-2	117										
IL-4	61	527	1044		8.6	17.0					
IL-5	121	417	953	3073	3.4	7.9	25	2.3	7.4		
IL-10	109	370	828	2927	3.4	7.6	27	2.2	7.9		
IL-12	96	364	768		3.8	8.0					
IL-13	106	404	1077	3900	3.8	10.2	37	2.7	9.7		
IFN _γ	91	329	699		3.6	7.7					
TNF _α	124	432	1009		3.5	8.1			2.3		
Expected	100	400	1000	4000	4.0	10.0	40.0	2.5	10.0	4.0	

Conclusions

1. A new sandwich ELISA based method has been evaluated and used to study the production of cytokines in this study.
2. The induction levels of eight cytokines have been quantified between two samples using the ELISA Array and a standard curve protocol in one experiment.
3. The ELISA Array can also be used to determine the ratio of induction levels among multiple samples in one experiment by using a four-parameter logistic-log model.

