

Multi-Analyte ELISArray™ Kits

Analyze 12 Cytokines or Chemokines Simultaneously



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Topics to be Covered

- **Who is SABiosciences?**
- **Challenges Facing Cytokine Researchers**
- **Solutions that the ELISArray Kits Provide**
- **How the ELISArray Kits Work**
 - Multi-Cytokine or Chemokine Flexibility
 - Best Performance, Ease of Use, Cost-Effectiveness
- **How YOU Can Use the ELISArray Kits**

Who is SABiosciences?



- Located in Frederick, Maryland, USA
- ~ 30 miles north of the NIH Bethesda campus
- Founded 10 years ago by NIH scientists
- Growth through new product development and product line extensions like the ELISArrays

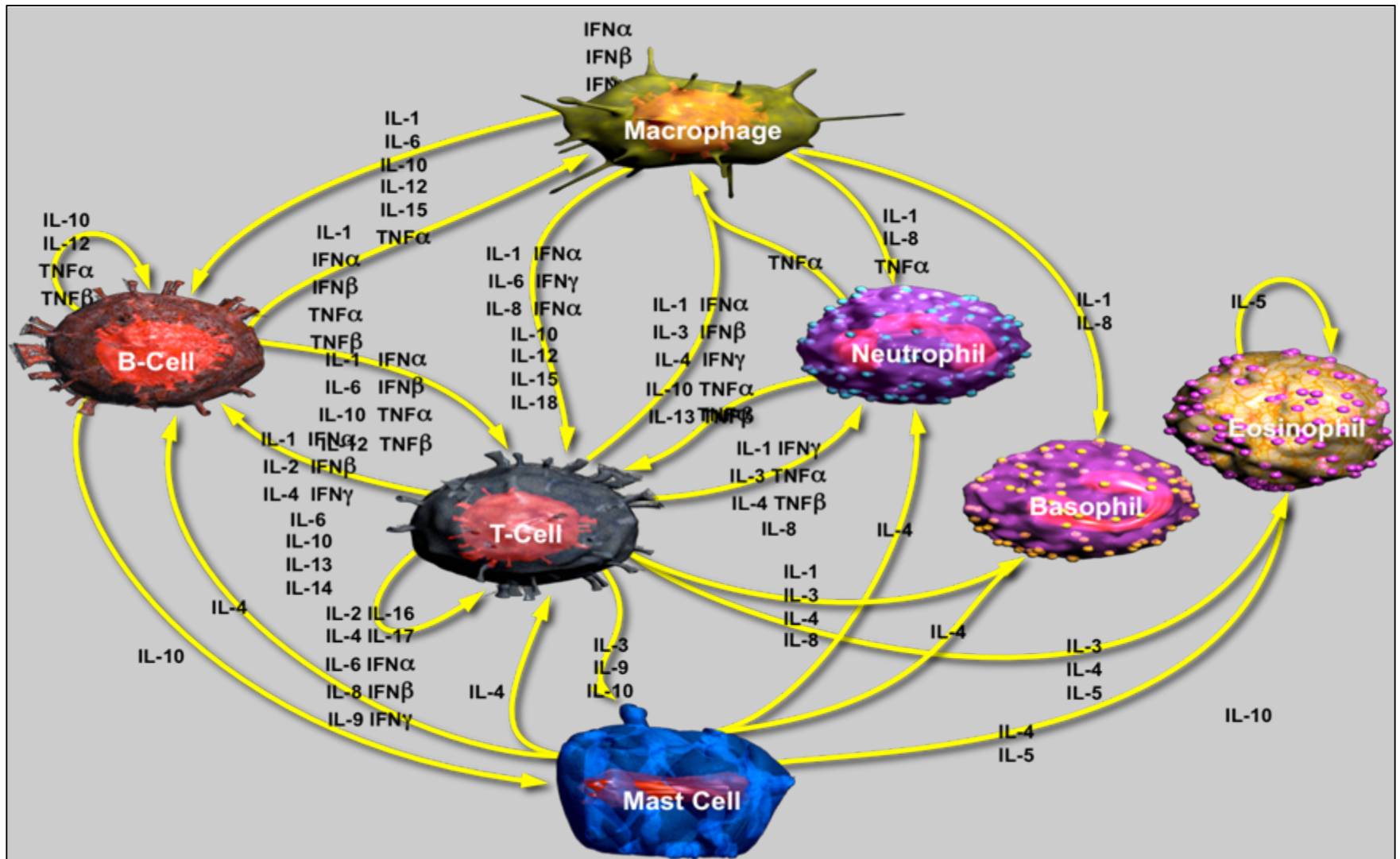
Systems Biology @ SABiosciences

Research	Technologies	SABiosciences Products
DNA ↓ Genomics & Regulation	Sequencing SNP Detection CHIP Methylation	CHIP-qPCR Assays Methyl-Profiler
RNA ↓ Gene Expression	Discovery Screening Confirmation	Gene Expression Service Core RT ² PCR Arrays, Oligo GEArrays RT ² miRNA Arrays and Assays RT ² qPCR Assays
Protein Gene Function Protein Function	Detection Knockdown Reporter System Phenotypes	ELISArray Kits PhosphoELISArray Kits siRNA Arrays / shRNA Plasmids Signal Reporter System

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Cytokine Signaling: A Complicated Network



Challenges Facing Cytokine Researchers

- **Common Mindset**
 - ELISA – Quantitative Protein Analysis
 - Only one cytokine at a time. What about others? Which ones?
 - Pick one & buy another ELISA kit
 - Money & time wasted if cytokine proves unimportant
- **Cost-Effective Multi-Analyte/Multiplex Analysis**
 - Format & pricing of ELISA products only analyze one cytokine
 - Multiplex methods require complicated & expensive equipment
- **Who do you believe?!**
 - ELISA Kits made by antibody manufacturers

What the ELISArray Kits Provide

- **Multi-Protein Flexibility:**

Profile twelve cytokines or chemokines in six samples at once using the same development / incubation time

All in one plate at the cost of one plate

- **High Performance with Minimal Effort:**

Profile with the highest levels of sensitivity and linearity possible with screened and validated capture and detection antibodies.

- **Ease of Use:**

No special equipment is required, only the ELISA plate reader already in your lab.

What is an ELISArray Kit?

- Simultaneously *profiles* multiple cytokines / chemokines
- Uses convenient & simple ELISA protocol
- COMPLETE kit with standardized set of Antigen Standards, Secondary Antibodies, Colorimetric Detection Reagents, Buffers

AND ...

- Twelve different eight-well strips
- Each strip is coated with one capture antibody
- All in one 96-well microtiter plate

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Multi-Analyte Flexibility: ELISArray Layout

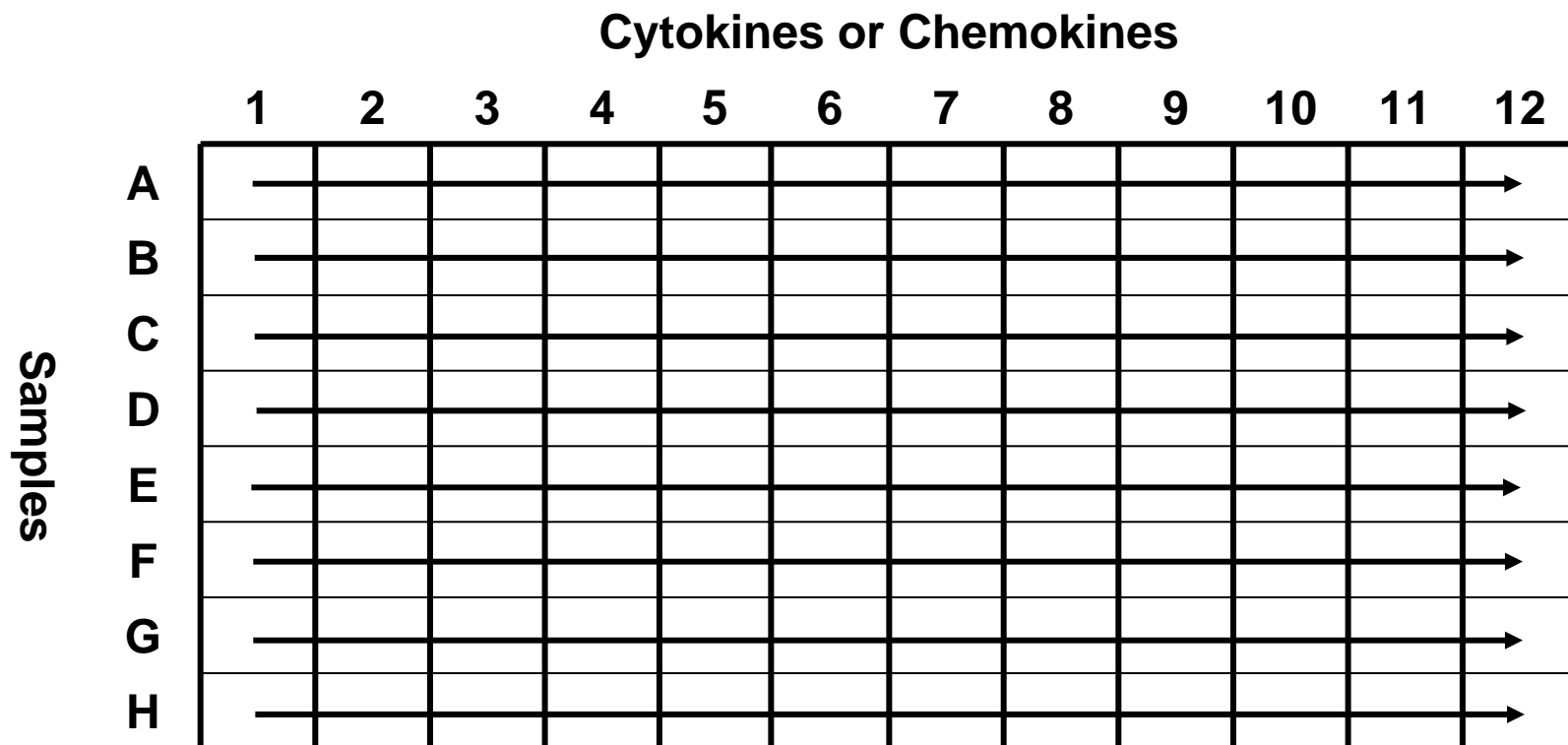
		Cytokines or Chemokines											
		1	2	3	4	5	6	7	8	9	10	11	12
Samples	A												
	B												
	C												
	D	IL1a	IL1b	IL2	IL4	IL6	IL8	IL10	IL12	IL17A	IFN γ	TNF α	GM-CSF
	E												
	F												
	G												
	H												

Each eight-well strip is coated with a capture antibody for a cytokine or chemokine. A set of twelve strips in one ELISArray represents twelve cytokines or chemokines. For example, Human Inflammatory Cytokines

Multi-Analyte Flexibility: ELISArray Content

- **New!!** TLR-induced Cytokines I: Viral-induced (Human & Mouse)
H TNF α , IL1b, IL6, IL12, IL17A, IL8, MCP-1, RANTES, IP-10, MIG, TARC, IFN α
M TNF α , IL1b, IL6, IL12, IL17A, SDF-1, MCP-1, RANTES, IP-10, MIG, TARC, 6Ckine
- **New!!** TLR-induced Cytokines II: Microbial-induced (Human & Mouse)
H TNF α , IL1b, IL6, IL12, IL17A, IL8, MCP-1, RANTES, MIP-1a, MIP-1b, MDC, Eotaxin
M TNF α , IL1b, IL6, IL12, IL17A, SDF-1, MCP-1, RANTES, MIP-1a, MIP-1b, MDC, Eotaxin
- **New!!** Common Chemokines (Human & Mouse)
H IL8, MCP-1, RANTES, MIP-1a, MIP-1b, IP-10, I-TAC, MIG, Eotaxin, TARC, MDC, GRO α
M RANTES, MCP-1, MIP-1a, MIP-1b, SDF-1, IP-10, MIG, Eotaxin, TARC, MDC, KC, 6Ckine
- Autoimmune Response (Human & Mouse)
H IL1b, IL4, IL6, IL10, IL12, IL17A, IFN γ , TNF α , TGF β 1, MCP1, MIP-1a, MIP-1b
M IL1b, IL4, IL6, IL10, IL12, IL17A, IFN γ , TNF α , TGF β 1, MCP1, MIP-1a, MIP-1b
- Common Cytokines (Human & Mouse)
H IL1a, IL1b, IL2, IL4, IL5, IL6, IL8, IL10, IL12A, IL13, IL17A, GM-CSF
M IL1a, IL1b, IL2, IL4, IL5, IL6, IL10, IL12A, IL13, IL17A, G-CSF, GM-CSF
- Inflammatory Cytokines (Human & Mouse)
H IL1a, IL1b, IL2, IL4, IL6, IL8, IL10, IL12, IL17A, IFN γ , TNF α , GM-CSF
M IL1a, IL1b, IL2, IL4, IL6, IL10, IL12, IL17A, IFN γ , TNF α , G-CSF, GM-CSF
- Th1 / Th2 / Th17 Cytokines (Human & Mouse)
H IL2, IL4, IL5, IL6, IL10, IL12, IL13, IL17A, IFN γ , TNF α , G-CSF, TGF β 1
M IL2, IL4, IL5, IL6, IL10, IL12, IL13, IL17A, IL23, IFN γ , TNF α , TGF β 1

Multi-Analyte Flexibility: ELISArray Layout



The lettered wells of each strip characterize the same biological sample. Each ELISArray microplate characterizes up to six samples.

Multi-Analyte Flexibility: ELISArray Layout

Cytokines or Chemokines

	1	2	3	4	5	6	7	8	9	10	11	12
Samples	A Negative Control (No Antigen)											
	B Experimental Sample 1											
	C Experimental Sample 2											
	D Experimental Sample 3											
	E Experimental Sample 4											
	F Experimental Sample 5											
	G Experimental Sample 6											
	H Positive Control (1 ng/ml each antigen)											

Each ELISArray microplate characterizes up to six samples:
One Negative Control, One Positive Control, Six Biological Samples

Ease of Use: Protocol Overview

1. Set up Antigen Standard (***included***) and sample dilutions.
2. Add assay buffer & samples to each ELISArray well.
Incubate 2 hours. Wash 3 ×.
Eight tubes – 8-channel pipettor
3. Add Detection Antibody Solution. Incubate 1 hour. Wash 3 ×.
Each diluted with the ***same*** volume of buffer.
Twelve tubes – 12-channel pipettor
4. Add Avidin - HRP. Incubate 30 minutes. Wash 4 ×.
5. Add Development Solution. Incubate ***15 minutes*** in the dark.
6. Add Stop Solution. Read OD450 within 30 minutes.

Screening Antibodies for Best Performance

- We systematically test every possible combination of capture & detection antibodies for each cytokine or chemokine using the following criteria:
 - **High Sensitivity**

The highest antigen concentration produces the maximum signal (OD450 ~ 2.0).
 - **Good Linearity**

The calibration curve is linear with a correlation factor R^2 greater than 0.950.
- A combination only passes if it meets these two criteria.

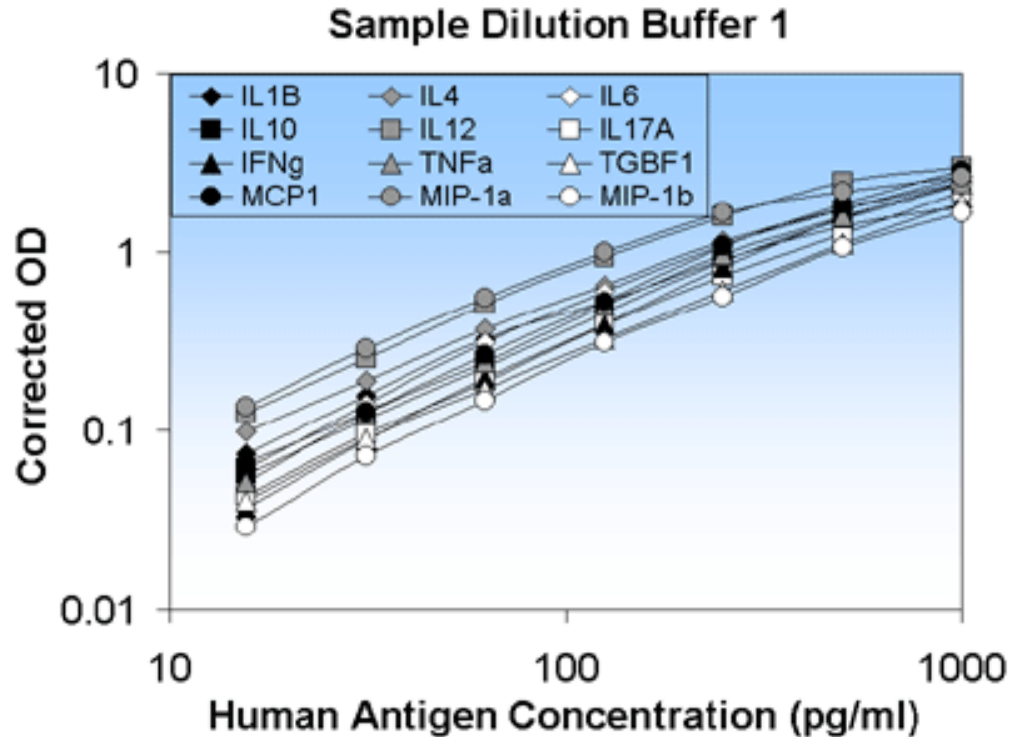
ELISArray Performance:

Optimize for Simultaneous Readout

EACH cytokine assay in the ELISArray set provides:

- The **SAME** linear response
- From the **SAME** antigen concentration range
- Using the **SAME** protocol
- Stated Limit of Detection (LOD) for each cytokine

Optimize for Simultaneous Readout



All 12 calibration curves obtained with same 15' development time
All 12 calibration curves are parallel

ELISArray Performance: Sensitivity

HUMAN				MOUSE			
Antigen	LOD	Antigen	LOD	Antigen	LOD	Antigen	LOD
IL1a	13.5	IL17A	28.4	IL1a	32.5	IL17A	46.2
IL1b	17.9			IL1b	55.2	IL23	67.2
IL2	35.1	IFN γ	19.6	IL2	24.2	IFN γ	39.0
IL4	7.0	TNF α	21.4	IL4	32.8	TNF α	30.5
IL5	14.0	GM-CSF	48.3	IL5	36.1	GM-CSF	23.7
IL6	14.0	G-CSF	20.9	IL6	58.8	G-CSF	25.6
IL8	16.5	MIP-1a	25.7			MIP-1a	35.1
IL10	5.9	MIP-1b	23.6	IL10	59.4	MIP-1b	26.7
IL12	12.6	MCP1	40.3	IL12	21.1	MCP1	25.5
IL13	15.0	TGF β 1	19.7	IL13	34.6	TGF β 1	22.9

Level of sensitivity typical of ELISA for these cytokines & chemokines
 LOD = Limit of Detection (pg/ml)

Why an ELISArray Kit?

You can believe us.

- SABiosciences is not dedicated to one antibody source
- Developed with the best possible antibody pairs available
- Take advantage of assay development & optimization expertise

You can profile more of the cytokines that you care about.

- Profile 12 cytokines in 6 samples with 1 kit
- Quickly & easily identify cytokines changing expression levels
- Follow up with an individual ELISA for *quantitative* results
- Corresponding Single Analyte ELISArray Kits also available

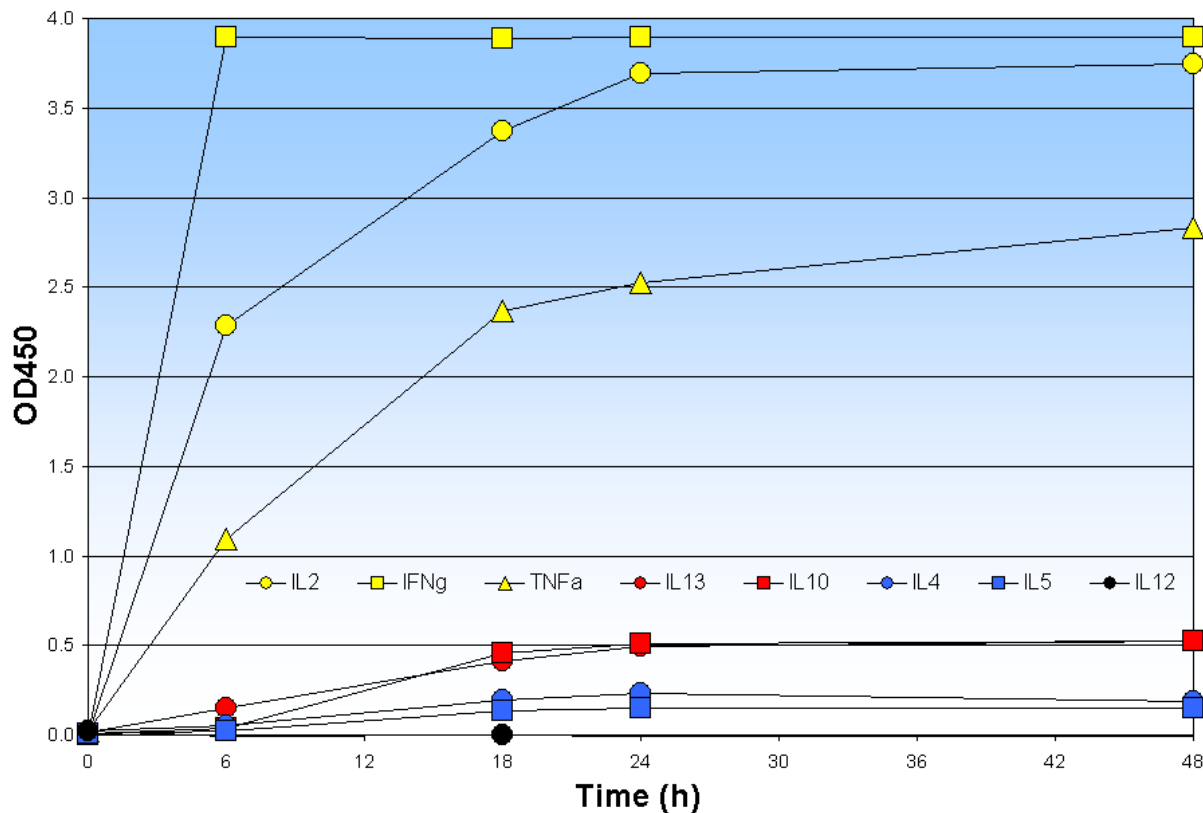
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ELISArray Kit Application Example

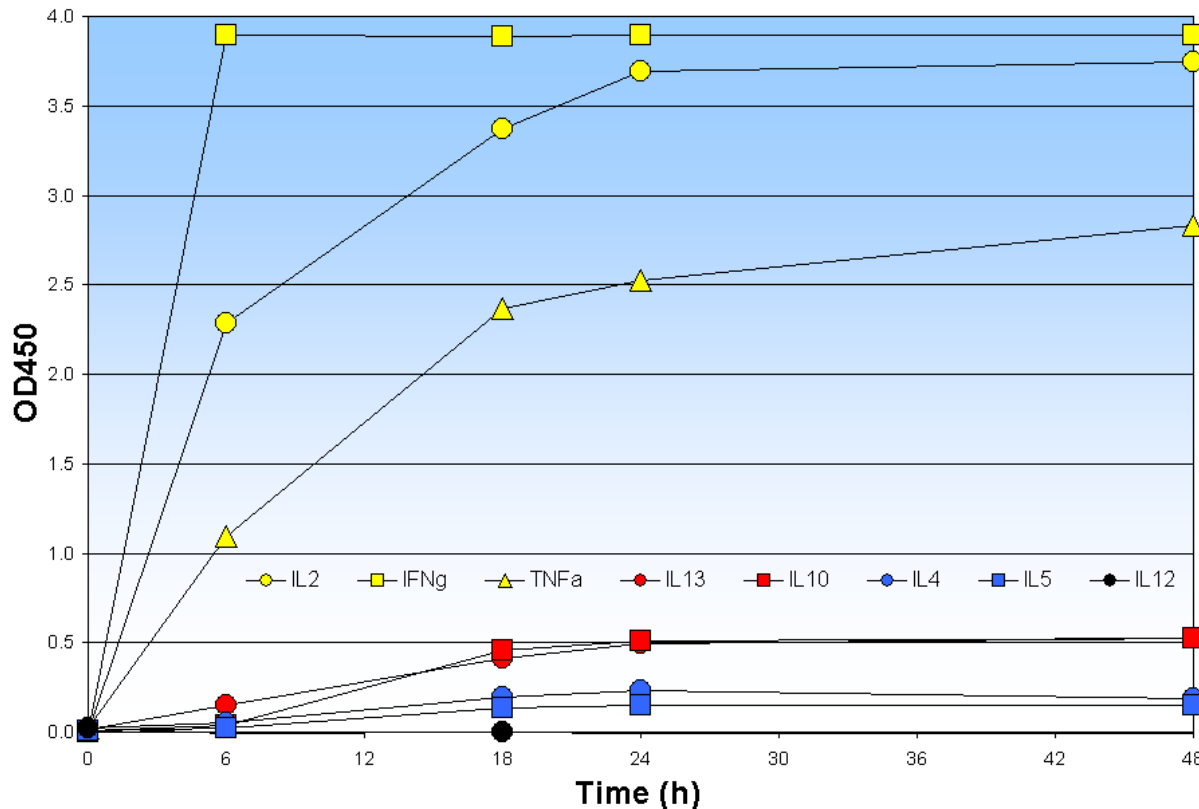
- Treat human peripheral blood mononuclear cells (PBMC) with PMA (50 ng/ml) and ionomycin (1 μ g/ml)
- Profile Th1 / Th2 cytokine induction over time: 0, 6, 18, 24, and 48 hours
- Follow up with Single Analyte ELISArray Kits for quantitative results

ELISArray Kit Application Example



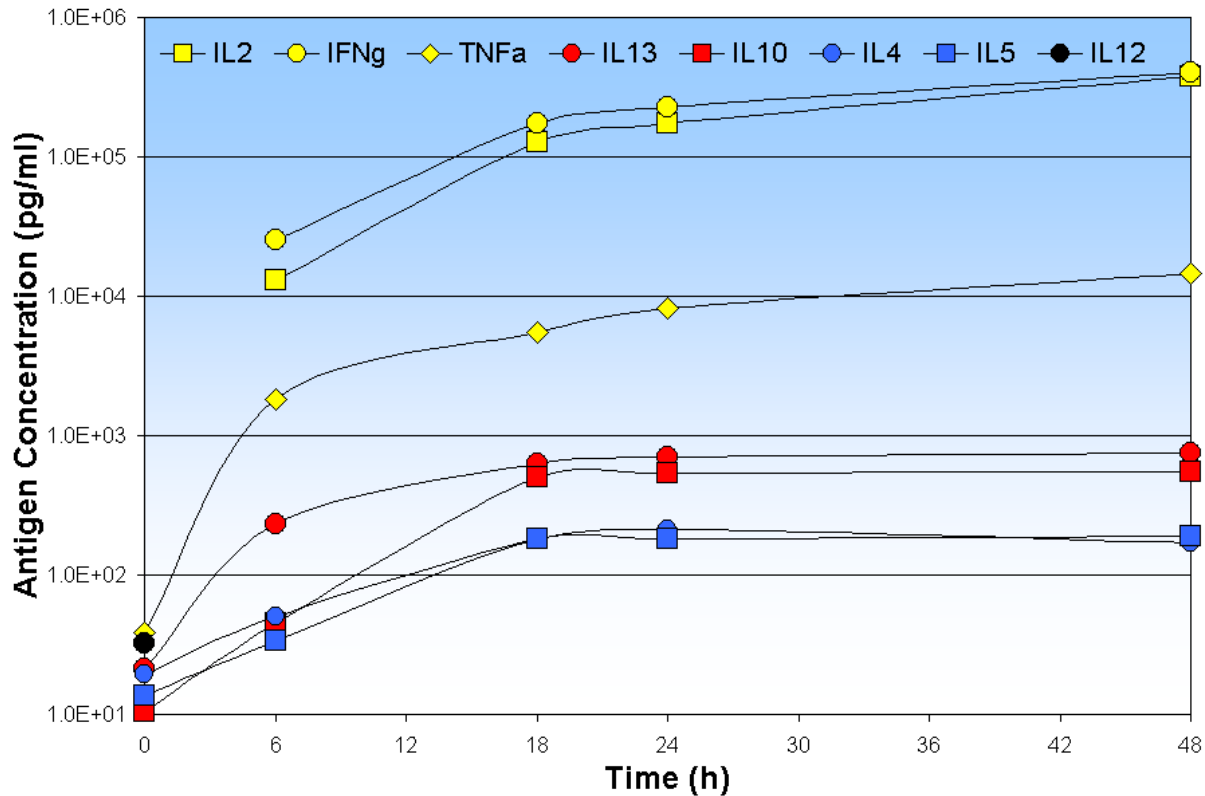
Large amounts of IL-2 and IFN γ are induced throughout, and apparently beyond, the time course tested. To a lesser extent, TNF α is induced throughout, and apparently beyond, the time course tested.

ELISArray Kit Application Example



Only more moderate amounts of IL4, IL5, IL10 and IL13 are induced, reaching a maximum at roughly just before 24 hours of stimulation, and even seem to start to decrease again at later times. The amount of IL12 is not detectable even after 48 hours of constant stimulation.

ELISArray Kit Application Example



Follow up with *quantitative* results from individual Single Analyte ELISArray Kits confirms the profiling results from the Multi-Analyte ELISArray Kits.

Available ELISArray Kits

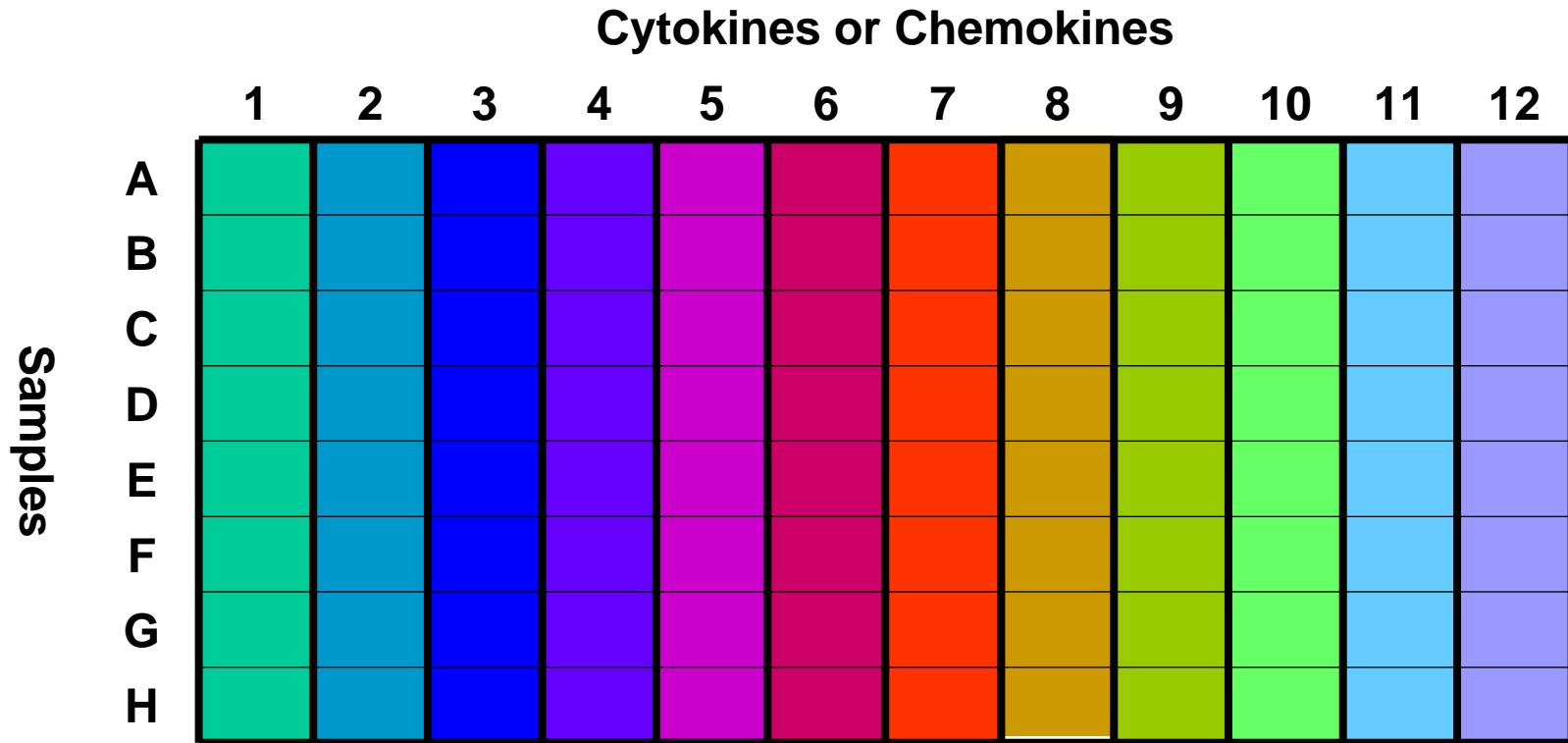
- **Multi-Analyte ELISArray Kits for Human and Mouse**
 - TLR-induced Cytokines I & II Common Chemokines
 - Autoimmune Response Common Cytokines
 - Inflammatory Cytokines Th1 / Th2 / Th17 Cytokines
- **Single Analyte ELISArray Kits for Human and Mouse**
 - New Mouse – RANTES, IP-10, SDF-1, MIG, Eotaxin, TARC, MDC, KC, 6Ckine
- **Mix-N-Match Flexibility:**
 - Define your own set of cytokine / chemokine 8-well strips
- **Off-the-Shelf Availability**

Mix-N-Match Flexibility

		Cytokines or Chemokines											
		1	2	3	4	5	6	7	8	9	10	11	12
Samples	A												
	B												
	C												
	D												
	E												
	F												
	G												
	H												

Set up your own combination of 12 cytokines or chemokines
OR set up replicate sets of a smaller number of analytes (2 x 6, 3 x 4)

Mix-N-Match Flexibility



Set up your own combination of 12 cytokines or chemokines
OR set up replicate sets of a smaller number of analytes (2 x 6, 3 x 4)

Mix-N-Match Flexibility

Cytokines or Chemokines

	1	2	3	4	5	6	7	8	9	10	11	12
A	Green	Green	Blue	Blue	Purple	Purple	Red	Red	Yellow	Yellow	Cyan	Cyan
B	Green	Green	Blue	Blue	Purple	Purple	Red	Red	Yellow	Yellow	Cyan	Cyan
C	Green	Green	Blue	Blue	Purple	Purple	Red	Red	Yellow	Yellow	Cyan	Cyan
D	Green	Green	Blue	Blue	Purple	Purple	Red	Red	Yellow	Yellow	Cyan	Cyan
E	Green	Green	Blue	Blue	Purple	Purple	Red	Red	Yellow	Yellow	Cyan	Cyan
F	Green	Green	Blue	Blue	Purple	Purple	Red	Red	Yellow	Yellow	Cyan	Cyan
G	Green	Green	Blue	Blue	Purple	Purple	Red	Red	Yellow	Yellow	Cyan	Cyan
H	Green	Green	Blue	Blue	Purple	Purple	Red	Red	Yellow	Yellow	Cyan	Cyan

Set up your own combination of 12 cytokines or chemokines
OR set up replicate sets of a smaller number of analytes (2 x 6, 3 x 4)

Mix-N-Match Flexibility

Cytokines or Chemokines

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Set up your own combination of 12 cytokines or chemokines
OR set up replicate sets of a smaller number of analytes (2 x 6, 3 x 4)

Cost-Effective Multi-Analyte Profiling

Minimum Initial Order	Price
One plate / One cytokine from another manufacturer	\$ 499
12 plates / 12 cytokines from another manufacturer	\$ 5988
One plate / One cytokine in ONE ELISArray Kit	\$ 349
One plate / Twelve cytokines in ONE ELISArray Kit Cataloged	\$ 449
(Custom) Mix-N-Match	\$ 499

Prices good for US Customers only.
Non-US customers should contact our local distributor.

SUMMARY

- **Multi-Protein Flexibility:**

Profile more of the cytokines / chemokines that you want to see

Don't just look at one-at-a-time

Four pre-designed sets of 12 human or mouse cytokines & chemokines

Mix-N-Match your own set of 12, or fewer in replicates

- **High Performance:**

Saved you time & effort by already screening for the best antibodies

Stated Limit of Detection, High Sensitivity, Good Linearity

Any cataloged or custom set uses the same protocol

- **Ease of Use:**

Conventional ELISA protocol and reader

No other special equipment needed

Multi-Analyte ELISArray™ Kit Technology Overview

Questions?

Contact Technical Support 9 AM – 6 PM EST

Telephone: 888-503-3187

Email: support@sabiosciences.com