



Cytokine Data Sheet

Offering Researchers Creative Solutions for Cytokine Screening

Cytokines play an important role in the understanding and treatment of diseases in many medical specialties.

Protein microarray technology is accelerating the rate at which researchers can obtain cytokine expression information. Utilizing FAST[®] Slides by Whatman, which are the world's premier protein arraying surface, quantitative MicroSpot ELISA kits and quantitative Cytokine Services offer researchers standardized solutions with unsurpassed technical expertise and support.

The simple, rapid assays offered by Whatman that utilize ELISA sandwich formats to measure concentrations of multiple cytokines from a single biological sample give researchers powerful, versatile and comprehensive solutions. The two assays are:

- FAST Macro, membrane-based antibody arrays are used to simultaneously evaluate the relative abundance of cytokines between different biological samples.
- FAST Quant[®], microspot ELISA for high throughput multiplex cytokine quantification, allows researchers to accurately quantify the concentration of several cytokines in dozens of biological samples simultaneously.

For researchers without the in-house capabilities to conduct these assays, Whatman offers a wide variety of cytokine array services. Working with the Whatman customer service center, researchers can create an array by selecting from our menu of antibodies of human and mouse cytokines. Using our FAST Quant system, our cytokine array processing and data analysis service offers researchers the results from the quantitative analysis of multiple cytokines.

FAST Macro

Membrane-Based Antibody Arrays

FAST Macro arrays are used to simultaneously evaluate the relative abundance of 20 different cytokines between different biological samples using chemiluminescent detection. FAST Macro offers researchers the following benefits:

- **High sensitivity** — The high binding capacity of the nitrocellulose membrane leads to a sensitivity as low as 15 pg/mL.

- **Reproducibility** — Cytokines are arrayed in triplicate with nine positive and three negative control spots per membrane.
- **Tested for cytokine specificity** — No cross reactivity.
- **Suitable for several sample types** — Can be used with serum, cell lysates and culture media.
- **No special equipment required** — No need for special instrumentation or software for detection and analysis.

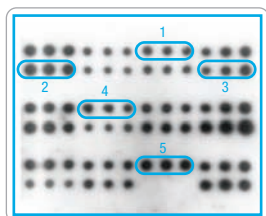
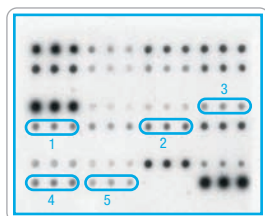
The FAST Macro Human I and Mouse I arrays consist of 20 anti-cytokine antibodies

arrayed on Whatman Protran nitrocellulose (BA-83, 0.2 μm), a surface which is well known for high protein binding capacity and excellent signal-to-noise ratios. Arrays are visualized using streptavidin/HRP-based chemiluminescent detection and x-ray film or a phosphor imager.

The FAST Macro kit includes either four or eight arrayed membranes, FAST Macro Wash and Blocking Buffers, and a biotinylated antibody cocktail.



FAST Macro: Membrane based antibody arrays are used to simultaneously evaluate the relative abundance of cytokines between different samples.



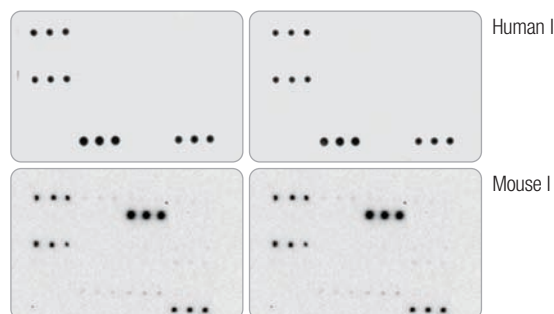
	Specificity	Sensitivity
1	IL-10	250 pg/ml
2	IP-10	150 pg/ml
3	IL-8	15 pg/ml
4	RANTES	40 pg/ml
5	TNF α	20 pg/ml

Human I

	Specificity	Sensitivity
1	GMCSF	31 pg/ml
2	IL-3	31 pg/ml
3	IL-4	125 pg/ml
4	IL-5	62 pg/ml
5	VEGF	62 pg/ml

Mouse I

Demonstration of Sensitivity: FAST Macro human and mouse membranes were incubated with an antigen cocktail consisting of all 20 recombinant human cytokine antigens. The concentration of each cytokine was equivalent to the sensitivity level of that cytokine.



Demonstration of Specificity and Reproducibility: To test for specificity, each of the 20 recombinant human and mouse cytokine antigens was individually incubated on a FAST Macro arrayed membrane and processed using all 20 biotinylated antibodies. In these examples, duplicate FAST Macro arrayed membranes were incubated with 1 ng/mL of TNF α antigen.



Comparisons of LPS-treated human THP-1 leukemia cells with untreated THP-1 cells in both cell lysate and cell culture supernatant using FAST Macro Human I microarrays: Human THP-1 leukemia cells were treated with 5 $\mu\text{g}/\text{mL}$ of lipopolysaccharide (LPS) for 6 hours. THP-1 cells cultured without LPS were used as a normal control. After 6 hours, the cells were pelleted and the cell culture supernatant was harvested for testing. The cell pellet was washed twice and then lysis buffer containing a protease inhibitor cocktail was added. After 15 minutes on ice, the lysed cells were spun and the supernatant (lysate) was harvested.

Samples were incubated with FAST Macro arrayed membranes and detected with chemiluminescence. FAST Macro Human I clearly demonstrates the induction of several cytokines after LPS treatment. By comparing the LPS treated to the untreated samples, 7 of the 20 screened cytokines are noticeably induced in the both the LPS stimulated cell lysate and cell culture supernatant. **Membrane 1** is the image from a 1:10 dilution of untreated lysate and **Membrane 2** is a 1:10 dilution of LPS-treated lysate. **Membranes 3 and 4** are images from undiluted, untreated cell culture supernatant and undiluted, LPS treated cell culture supernatant, respectively. The THP-1 lysates and cell culture supernatants were also tested by ELISA using undiluted samples. The following four cytokines' concentrations were determined: IL1 α , IL6, IL8, and TNF α . The ELISA results supported the FAST Macro Human I data. For example, IL8's concentration, as determined by ELISA, is 30 pg/mL for untreated supernatant, 170 pg/mL for untreated lysate, 9,370 pg/mL and 11,000 pg/mL for LPS treated supernatant and lysate respectively.

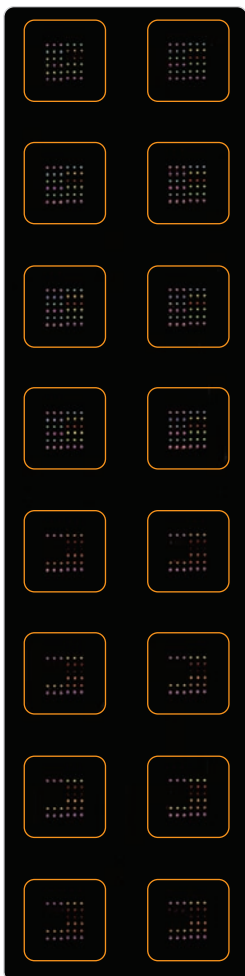
FAST Quant

MicroSpot Arrays for Cytokine Quantification

FAST Quant brings together the power of array technology with the quantitative nature and high-throughput capabilities of traditional ELISA. This array boasts the following features:

- **Saves time and money** — Multiplexing speeds up processing time of samples
- **Requires very low sample per array volumes** — As little as 35 μ L of sample per array are necessary.
- **Over 500 quantified answers** — 56 samples plus 8-point standard curve on 64 pre-spotted arrays.
- **Four human and two mouse panels.**
- **Developed in accordance with ICH and FDA guidelines for immunoassays.**

Each FAST Quant Kit includes four 16-pad FAST Slides. Each pad is pre-arrayed with a panel of Cytokine antibodies. Complete seal is achieved without adhesive.



FAST Quant represents a quantum leap forward in protein microarray technology.

With FAST Quant, a researcher can accurately determine the concentration of several cytokines in dozens of biological samples simultaneously, using familiar ELISA immunochemistry.

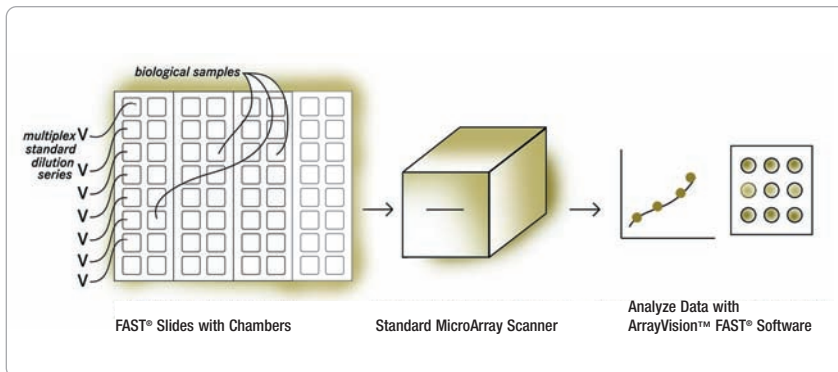
Each FAST Quant kit contains 64 arrays of eight to ten monoclonal antibodies with affinities for common human or mouse cytokines.

The antibodies are arrayed in a quantitative fashion in triplicate on each array.

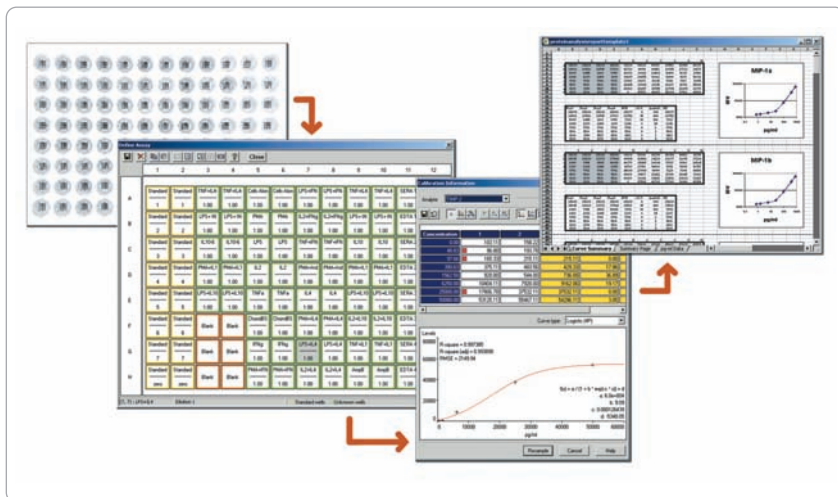
Using four 16-pad FAST Slides placed in a FAST Frame, FAST Quant offers an 8 x 12 cm footprint — the same as the traditional microplate format.

Because of the solid-phase nature of a microspot assay, it is not necessary to take duplicate measurements of each sample. The MicroSpot ELISA reaction is largely concentration dependent, unlike an ELISA where the reaction is both concentration and volume dependent.

The left image shows a 16-pad FAST Slide scan. The orange squares indicate the pad positions on the slide.



Sample processing: Make dilution series from standards provided. Incubate curve on eight of the arrays and use the remaining arrays for unknown samples. Collect data using any standard microarray scanner and quantify data with e.g. ArrayVision™ FAST® software.



Data analysis: FAST Quant data can be analyzed directly after scanning to provide quantitative results. ArrayVision FAST software includes templates designed to fit FAST Quant, including standard curves.

FAST Quant ranges and sensitivity: Example: FQ Human Th1/Th2 LOD calculated as 2 standard deviations above zero antigen controls.

Cytokine	IL-1 β	IL-2	IL-4	IL-5	IL-6	IL-10	IL-13	FN γ	TNF α
Range (pg/mL)	2.4 – 2500	3.8 – 2500	8.2 – 2500	13 – 2500	3.3 – 2500	50.6 – 12500	90.7 – 12500	14.6 – 12500	5.1 – 2500
LOD (pg/mL)	2.4	3.8	8.2	13	3.3	50.6	90.7	14.6	5.1

Cytokine Assay

Development, Processing and Data Analysis Service

Using cutting edge, automated microarray technologies and innovative approaches, the Cytokine Array Development, Processing and Data Analysis Service allows researchers to acquire distinct, reliable scientific data from the proteomic specialists at Whatman. Researchers who use our service enjoy the following benefits:

- **Reduces costs** — Saves time and money due to the cost of antibodies, capital equipment, assay development and processing, lab personnel, software acquisition and analysis.
- **Standardizes studies** — Assays are processed and analyzed by expert staff in a dedicated array processing facility.

To take advantage of this service, simply select from the Whatman cytokine antibody menu of 54 human and 19 mouse specificities. New antibodies can be sourced as necessary. Be sure to preserve your valuable samples. With

our high-throughput ELISA format, as little as 100 μ L of human sample per array is necessary. Applicable sample types are serum, plasma, cell culture supernatants, cellular extracts and wound effluent.

Whatman supplies the following data to the researcher:

- Total fluorescent signal per microspot
- Standard curves per cytokine
- % CV
- Standard deviation
- pg/mL value per sample
- Access to raw and trimmed data

Human Cytokines available	Sensitivity (pg/mL)	Dynamic range (pg/mL)	Dose Response Slope	Mouse Cytokines available	Sensitivity (pg/mL)	Dynamic range (pg/mL)	Dose Response Slope
Angiogenin	30	30–1,000	0.79	GM-CSF	1	1–300	0.81
Angiopoietin-2	3	3–300	0.31	IFN γ	3.2	6–400	1.3
Collagen Type 1	1,000	1000–100,000	0.60	IL-1 β	3	3.1,000	0.73
Collagen Type IV	30	30–30,000	0.60	IL-2	3	3–1,000	0.82
EGF	1	1–30	0.87	IL-3	3	3–1,000	0.90
Eotaxin	3	3–300	0.69	IL-4	3	3–1,000	0.83
FAS	3	3–10,000	0.52	IL-5	3	3–300	1.6
FAS Ligand	3	3–3,000	0.70	IL-6	3	3–1,000	0.8
FGF-basic	10	10–1,000	0.82	IL-10	3.2	24–20,000	1.2
Fibronectin	10	10–10,000	0.48	IL-12p40	3.2	3.2–400	0.76
Fractalkine	100	100–30,000	0.77	IL-12p70	3.2	3.2–400	1.1
GM-CSF	1	1–400	0.78	IL-13	3.2	24–20,000	1.1
ICAM-1	100	100–3,000	0.97	MCP-5	3	3–300	0.81
IFN γ	10	10–3,000	0.58	M-CSF	3	3–1,000	0.82
IGF-1	10	10–1,000	0.62	MIP-1 α	10	30–1,000	1.2
IGF-II	30	100–100,000	0.79	MIP-2	1	3–1,000	0.93
IL-1 α	1	1–300	0.84	RANTES	10	10–1,000	0.74
IL-1 β	3	3–1,000	0.82	TNF α	7	7–1,000	0.77
IL-2	3	10–1,000	0.88	VEGF	3.2	32–2,000	1.1
IL-3	3	3–300	0.79				
IL-4	3	10–3,000	0.84				
IL-5	10	10–3,000	1.11				
IL-6	3	10–3,000	0.87				
IL-6-R	1	1–1,000	0.77				
IL-7	1	1–100	0.92				
IL-8	3	3–3,000	1.1				
IL-10	30	100–3,000	0.68				
IL-12p40	30	30–10,000	0.85				
IL-12p70	30	100–10,000	1.0				
IL-13	100	100–3,000	0.69				
IL-17	3	3–1,000	0.70				
IP-10	30	30–1,000	0.55				
KGF	10	10–1,000	0.76				
MCP-1	1	3.2–400	0.90				
MCP-2	1	1–300	0.74				
MCP-3	1	1–300	0.87				
MCP-4	3	3–300	0.88				
M-CSF	3.2	3.2–400	0.75				
MIF	3	3–10,000	0.75				
MIP-1 α	16	16–400	0.76				
MMP-1	10	10–10,000	0.83				
MMP-9	3	3–10,000	0.57				
PDGF-AA	100	300–500,000	0.50				
PDGF-BB	10	10–300	1.0				
RANTES	3.2	3.2–400	0.90				
SDF 1	100	100–30,000	0.67				
TGF α	1	1–1,000	0.61				
TGF β	30	100–10,000	0.93				
TIMP-1	3	3–1,000	0.88				
TNFR1	3	3–10,000	0.59				
TNFRII	50	100–30,000	0.63				
TNF α	3	3–1,000	0.93				
Tpo	30	30–1,000	1.5				
VEGF	50	100–30,000	0.96				



FAST Quant

Processing and Data Analysis Service

Using Whatman FAST Quant standard kits, the FAST Quant Processing and Data Analysis Service offers researchers results from the quantitative analysis of multiple cytokines processed in a FAST Slide-based, MicroSpot ELISA format. The service includes array processing and data analysis. Customer-supplied biological samples are screened against the selected FAST Quant kit menu to determine the quantitative

measurements. Researchers who use our service realize the following benefits:

- **Reduces costs** — Expenditures for capital investment, and assay processing and analysis are reduced.
- **Standardizes studies** — Assays are processed and analyzed by expert staff in a dedicated array processing facility.

To use this service, simply select from the Whatman FAST Quant kit menu of four human and two mouse panels. Be sure to preserve your valuable samples. With our high-throughput ELISA format, as little as 100 μ L of human sample per array is

necessary. Applicable sample types are serum, plasma, cell culture supernatants, cellular extracts and wound effluent. Whatman supplies the following data to the researcher:

- Total fluorescent signal per microspot
- Standard curves per cytokine
- % CV
- Standard deviation
- pg/mL value per sample
- Access to raw-trimmed data.



Ordering Information

FAST Macro			
Description / Content	# of membranes / # of arrays	Qty/Pkg	Item #
FAST Macro Human I Kit 4	4 / 4	1	10486151
FAST Macro Human I Kit 8	8 / 8	1	10486152
Content: Eotaxin, GM-CSF, IFN γ , IL-1 α , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IP-10, MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 α , RANTES, TNF α			
FAST Macro Mouse I Kit 4	4 / 4	1	10486166
FAST Macro Mouse I Kit 8	8 / 8	1	10486167
Content: MCSF, GM-CSF, IFN γ , IL-3, IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-12p40, IL-10, IL-13, IL-12p70, MCP-1, MCP-5, MIP-2, VEGF, MIP-1 α , RANTES, TNF α			
FAST Quant			
Description / Content	# of membranes / # of arrays	Qty/Pkg	Item #
FAST Quant Human Th1/Th2	4 / 64	1	10486031
Content: IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IFN γ , TNF α			
FAST Quant Human II	4 / 64	1	10486060
Content: IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, GM-CSF, RANTES, MCP-1			
FAST Quant Human Angiogenesis	4 / 64	1	10486063
Content: Angiogenin, Angiopoietin-2, PDGF-BB, VEGF, FGF- β , KGF, TIMP-1, ICAM-1			
FAST Quant Human Chemokine	4 / 64	1	10486064
Content: Eotaxin, RANTES, MCP-1, MCP-2, MCP-3, MCP-4, IL-8, IP-10, MIP-1 α			
FAST Quant Mouse Th1/Th2	4 / 64	1	10486061
Content: IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IFN γ , TNF α			
FAST Quant Mouse II	4 / 64	1	10486062
Content: IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12p70, GM-CSF, RANTES, IFN γ			
Accessory			
Description / Content		Qty/Pkg	Item #
FAST Frame Multi-Slide Plate — holds up to 4 FAST Slides		1	10486001

With FAST Macro, FAST Quant, and the Cytokine Services Whatman offers a comprehensive solution for cytokine screening.

FAST Macro offers a human antibody panel and a slightly different mouse panel to screen for 20 different cytokines. FAST Quant is available in 6 different standard versions to quantify 8 to 10 different cytokines per sample in parallel. Cytokine Services meet the needs of customers who want to send in samples and get back reliable, standardized results.

Please contact your local Whatman office to discuss the Cytokine Array Development, Processing and Data Analysis services you require.

Whatman Quality

Whatman is a global leader in separations technology and is known in the scientific community for providing Innovative Life Science products and solutions. Our instinct for simplification accelerates the rate of discovery, reduces costs and saves time.

For more information, visit www.whatman.com

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