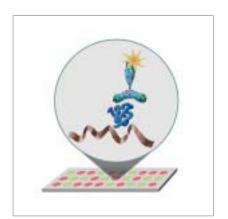




Reverse Arrays



Protein Binder Arrays

Protein microarrays are tools that can be used in many different areas of research, including basic and translational research. Protein arrays can take on many different formats and can be used to do more than simple expression profiling of samples.

Recent publications have demonstrated that protein microarrays can be used entire proteomes for new proteins and profile hundreds of patient samples their own arrays, as well as off-the-shelf arrays and protein array services.

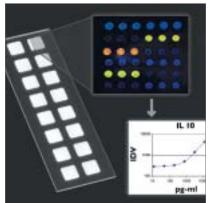


Proteomic arrays are typically high-density arrays (>1000 elements/array) that are used to identify novel proteins or protein-protein interactions. The library that is arrayed can come from many possible sources, including expression libraries, and can contain known, as well as unknown, elements. The sample to probe the array can come from virtually any source.

To detect proteins that are bound to the array, the samples must be labeled directly with a fluorophore or a hapten. Alternatively, in some applications, antibodies can be used to detect binding events. One common use is for antibody screening.

#### Microspot ELISA and Antibody Arrays

Microspot ELISA and antibody arrays are used for quantitative profiling of protein expression in cell cultures or clinical specimens. Typically these arrays are low-density (9-100 elements/array). In these arrays, known antibodies are arrayed and used to capture antigens from unknown samples. To detect antigen that is bound to the array, the antigen either needs to be labeled directly with a fluorophore, or a second binder/antibody can be used. The latter option creates a sandwich assay similar to a traditional ELISA, only in a microspot format.





Proteomic Arrays



Microspot ELISA and Antibody Arrays

#### Single-Capture Antibody Arrays

Single-capture antibody arrays consist of multiple, known antibodies arrayed to a solid surface and used to profile the presence of specific antigens from a pooled sample, usually consisting of both a normal and disease-present sample. A single capture antibody array utilizes a direct or hapten labeling system, which does not require a matched antibody. Single-capture antibody arrays offer a qualitative profiling tool to detect binding events. The Whatman Serum Biomarker Chip offers an example of a single-capture antibody array.

#### Reverse Arrays

Reverse arrays are used to profile dozens or hundreds of samples (research or clinical) for the presence of a small number of antigens (1-3). Cell lysates, material from laser capture microdissection, or serum samples, are arrayed. This creates an array of 'unknowns' that can be probed with a small number of antibodies. Visualization can be performed with a detection or 'top' antibody linked to a fluorophore or color detection reagent.

#### **Protein Binder Arrays**

Protein arrays can be used to identify novel protein binding motifs or protein-protein interactions. Engineered or synthetic proteins, or peptides with various binding motifs are arrayed, and the array is probed with complex protein samples. Detection with a known antibody allows the researcher to identify previously unknown binding events.

simultaneously. Whatman has led the way in protein array technology starting with the development of the FAST Slide: the premier protein arraying surface. We now offer kits, reagents and protocols for scientists who wish to develop



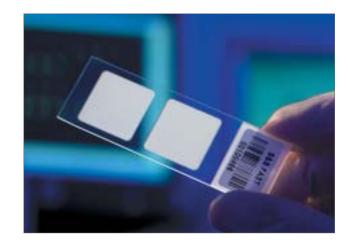
## FAST® Slides

#### Protein Microarray Surface

FAST Slides are the premier surface for protein microarray applications.

FAST Slides are glass slides coated with a proprietary nitrocellulose polymer. The polymer binds proteins in a non-covalent, irreversible manner and can be probed using the same method as in traditional blots.

The three-dimensional surface of a FAST Slide maintains reactivity of proteins and gives excellent reproducible results. It is usable with fluorescent, chemiluminescent or radiographic detection systems and is compatible with microarray scanners and robots.



Perhaps the most significant advantage of FAST Slides over modified glass surfaces is that the matrix retains arrayed proteins in near-quantitative fashion. This property translates into antibody arrays with unparalleled sensitivity down to antigen concentrations of 1 pg/mL. These qualities make FAST Slides the most reliable surface for microarray experiments and provide a level of confidence that no other surface has.

#### **Features**

- Superior protein binding capacity
- · Highest sensitivity and dynamic range
- Excellent long-term stability of printed proteins
- Compatible with all detection methodologies
- Compatible with commercially available arraying robots

FAST Slides are suitable for many types of protein microarrays including antibody arrays and microarray Westerns. There are tremendous advantages to using FAST Slides over traditional ELISAs and Westerns including less sample required, better sensitivity, linearity and quantitation. The largest advantage is hundreds or thousands of antibodies or samples can be screened simultaneously.

#### Microarray Westerns

An alternative strategy for protein microarrays is to array samples containing multiple proteins on the FAST Slide and probe with labeled antibody or set of antibodies. The advantage of the micro format is that extracts from various treatments and time points can be arrayed on the same slide. Once arrayed, the levels of multiple proteins can be measured and compared simultaneously.

Description
Up to 10,000 spots (150 µm spot size, 300 µm pitch)
Bar-coded
2x up to 3600 spots (150 μm spot size, 300 μm pitch)
Bar-coded
Pad spacing: 9 mm (microplate spacing)
8x up to 256 spots (150 µm spot size, 300 µm pitch)
Not bar-coded
Pad spacing: 9 mm (microplate spacing)
16x up to 256 spots (150 µm spot size, 300 µm pitch)
Not bar-coded

Ordering Information - FAST Slides					
Description	Pad Dimensions (mm)	Quantity/Pack	Catalog Number		
FAST Slides - 1-pad *	20 x 51	20	10 484 182		
FAST Slides - 2-pads *	20 x 20	10	10 485 317		
FAST Slides - 8-pads	6 x 6	10	10 485 320		
FAST Slides - 16-pads	6 x 6	10	10 485 323		

<sup>\*</sup> Bar-coded

## FAST® PAK

#### **Protein Array Kits**

FAST PAK protein array kits provide the necessary components for researchers to conveniently build and process protein microarrays.

FAST PAK kits are available for all FAST Slide formats (1-, 2-, 8- and 16-pads) and include protein arraying buffer, protein array blocking buffer, protein array wash buffer and incubation chambers.



40 FAST Slides FAST PAK

#### Features and Benefits

- 1-, 2-, 8- or 16-array pads on each slide
- Each pad can be processed separately to increase the number of arrays on each slide and reduce sample volume
- · Ideal for multiplex experiments, side-by-side comparisons and control experiments all on the same slide
- · Reproducible results from slide-to-slide and pad-to-pad
- Increased protein stability and enhanced signal intensity with FAST PAK array buffer
- · Proprietary protein array blocking buffer ensures optimum signal-to-noise ratios and promotes specific binding
- · Flexibility for detection by fluorescent, chemiluminescent, colorimetric and radiographic methods

#### **Applications**

- ELISA format (sandwich assay) experiments using antibody arrays
- Reverse phase (micro-Western) arrays using cell or tissue lysates
- Purified protein arrays
- Carbohydrate arrays
- Lipids and other materials which can be arrayed on nitrocellulose

Description	Catalog Number
FAST PAK, 1-pad (original)	10 485 262
10 FAST Slides, 10 Incubation Chambers, 40 Chamber Covers, 10 mL 2x Protein Arraying Buffer,	
15 mL Protein Array Blocking Buffer, 125 mL 10x Protein Array Wash Buffer	
FAST PAK, 2-pad	10 485 319
10 FAST Slides, 10 Incubation Chambers, 40 Chamber Covers, 10 mL 2x Protein Arraying Buffer,	
15 mL Protein Array Blocking Buffer, 125 mL 10x Protein Array Wash Buffer	
FAST PAK, 8-pad	10 485 322
10 FAST Slides, 10 Incubation Chambers, 40 Chamber Covers, 10 mL 2x Protein Arraying Buffer,	
15 mL Protein Array Blocking Buffer, 125 mL 10x Protein Array Wash Buffer	
FAST PAK, 16-pad	10 485 325
10 FAST Slides, 10 Incubation Chambers, 40 Chamber Covers, 10 mL 2x Protein Arraying Buffer,	
15 mL Protein Array Blocking Buffer, 125 mL 10x Protein Array Wash Buffer	
FAST Frame for 4 slides	10 486 001
Chip Clip for 1 slide	10 486 081

(Detection reagents must be provided by the end-user)

For the latest protocols visit www.arraying.com

# FAST Quant® System

#### MicroSpot ELISA for High-Throughput Multiplex Cytokine Quantification

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.

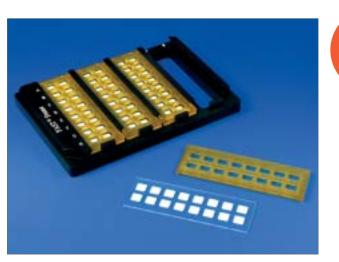
Built on FAST technology, the high protein binding capacity surface chemistry, FAST Quant combines the power of array technology with the quantitative nature and high-throughput capabilities of traditional ELISA. FAST Quant exhibits sensitivity and reproducibility better than traditional ELISA.

Each FAST Quant kit contains 64 arrays of 8-10 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 (sold separately) FAST Quant offers an 8 x 12 cm footprint - the same as the traditional microplate format. A standard curve can be generated by creating a dilutional series from recombinant antigen mass standards. Due to the solid-phase nature of a microspot assay, it is not necessary to take duplicate measurements of each sample. The MicroSpot ELISA reaction is entirely concentration dependent, unlike an ELISA where the reaction is both concentration and volume dependent.



FAST Quant System



FAST Frame

FAST Quant antibody arrays offer a variety of human and mouse menus. All arrays come in a four-slide kit with recombinant antigens standard, detection antibodies and processing buffers.

Data analysis is seamless using data reduction software, ArrayVision FAST, the cutting edge utility for protein array image analysis. The software provides rapid data acquisition and comprehensive reports. ArrayVision analyzes any .tif image from virtually all commercial imaging instruments. The application provides standard curve data, concentrations of unknowns, and percent coefficient of variation for each analyte. FAST Quant is another clear example of Whatman commitment to providing the scientific community with the best solution for multiplex cytokine measurements.

42 FAST PAK FAST PAK

Ordering Information - FAST Quant System			
Description	Catalog Number		
FAST Quant Human Th1/Th2	10 486 031		
FAST Quant Mouse Th1/Th2	10 486 061		
FAST Quant Human II	10 486 060		
FAST Quant Mouse II	10 486 062		
FAST Quant Human Angiogenesis	10 486 063		
FAST Quant Human Chemokine	10 486 064		
FAST Frame Slide Holder (4 slides)	10 486 001		
Chip Clip Slide Holder (single slide)	10 486 081		

Each kit includes:

Four 16-pad FAST Slides; each pad is pre-arrayed with a panel of cytokine antibodies (choice of six panels)

Four silicon 16 well chambers

Recombinant antigen standard cocktail for dilutional series (standard curve)

Biotinylated detection antibody cocktail

Whatman Protein Array Buffer, Protein Array Wash Buffer and Protein Array Blocking Buffer

## Human and Mouse Panels

## FAST Quant® Systems

FAST Quant antibody arrays offer a variety of human and mouse menus. All arrays come in a four-slide kit with recombinant antigens standard, detection antibodies and processing buffers.



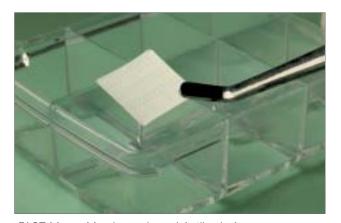
FAST Qu	ant Human	and N	Mouse Menus				
			Catalog Number				Catalog Number
FAST Quant H	uman Th1/Th2		10 486 031	FAST Quant	Mouse Th1/Th2		10 486 061
Cytokines commonly associated with the		Cytokines co	ommonly associa	ted with the			
Th1/Th2 immu	ne response sys	tem		Th1/Th2 imn	nune response sy	/stem	
IL-1b	IL-5	IL-13		IL-1b	IL-5	IL-13	
IL-2	IL-6	TNFα		IL-2	IL-6	TNFα	
IL-4	IL-10	IFNγ		IL-4	IL-10	IFNγ	
FAST Quant H	uman II		10 486 060	FAST Quant	Mouse II		10 486 062
IL-1b	IL-10	-		IL-1b	IL-12p70	-	
IL-2	IL-12p70	-		IL-2	GM-CSF	-	
IL-4	GM-CSF	-		IL-4	RANTES	-	
IL-8	RANTES	-		IL-6	IFNγ	-	
IL-6	MCP-1	-		IL-10	-	-	
FAST Quant H	uman Angiogene	esis	10 486 063	FAST Quant	Human Chemoki	ine	10 486 064
PDGF-BB	KGF	-		Eotaxin	MCP-4	-	
VEGF	TIMP-1	-		RANTES	IL-8	-	
FGFβ	ICAM-1	-		MCP-1	IP-10	-	
Angiogenin	Angiopoietin-2	2 -		MCP-2	MIP-1α	-	
-	-	-		MCP-3	-	-	

## FAST® Macro

#### Membrane-based Antibody Arrays

Whatman FAST Macro membrane-based antibody arrays are used to simultaneously evaluate the relative abundance of 20 different cytokines between different biological samples, such as disease state versus normal state, using chemiluminescent detection.

The FAST Macro Human I and Mouse I arrays consist of 20 anti-cytokine antibodies arrayed on Whatman Protran nitrocellulose (BA-83, 0.2  $\mu m)$ , a surface which is well known for high protein binding capacity and excellent signal-to-noise ratios.



FAST Macro Membrane-based Antibody Array

Designed as a screening tool to compare relative expression levels of cytokines/growth factors in different samples, one human panel and one mouse panel are included. The FAST Macro Kit includes either four or eight arrayed membranes, FAST Macro Wash and Blocking Buffers, and a biotinylated antibody cocktail. Arrays are visualized\* using streptavidin/HRP-based chemiluminescent detection and x-ray film or a phosphor imager.

44 FAST Quant System FAST Macro 45

FAST Macro is an inexpensive way to discover the power of multiplex antibody arrays with sensitivity as low as 15 pg/mL that can be used with serum, cell lysates and culture media samples. Results are comparable to ELISA results. No special instrumentation or software is required for detection and analysis.

\* Detection reagents must be provided by the end-user.

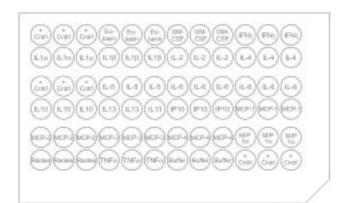
#### Features

- Requires as little as 650 µL of sample
- Array-to-array reproducibility
- Cytokines are arrayed in triplicate with 9 positive and 3 negative control spots per membrane
- Tested for cytokine specificity virtually no cross reactivity
- Membrane is notched in one corner for orientation
- Cytokine spots are visible on the unprocessed membrane - inert dye disappears during membrane wet-out

Samples as small as 400  $\mu$ L can be processed using the optional Whatman Chip Clip in conjunction with a Whatman dual-well array incubation chamber and a standard, untreated glass microscope slide.



FAST Macro Kit



FAST Macro Human I Array Map

Ordering Information - FAST Macro			
Description	Quantity/Pack	Catalog Number	
Membrane-Based Microarrays			
FAST Macro Human I Kit (4 arrays)	1	10 486 151	
FAST Macro Human I Kit (8 arrays)	1	10 486 152	
FAST Macro Mouse I Kit (4 arrays)	1	10 486 166	
FAST Macro Mouse I Kit (8 arrays)	1	10 486 167	
Chip Clip Slide Holder	1	10 486 081	
Dual-Well Array Incubation Chambers	10	10 486 087	

# Serum Biomarker Chip

### **High-Density Profiling**

The Serum Biomarker Chip enables proteomics researchers to profile and pattern the molecular signature of human serum. The chip addresses the need for a high-throughput technology to enable research in the fields of risk stratification, disease prognosis, drug eligibility, prediction of safety and efficacy, and therapeutic monitoring.

The Serum Biomarker Chip is a single-capture antibody array built on the FAST Slide dual-pad platform. Each slide has two identical arrays of antibodies printed in triplicate. Two-color fluorescent detection permits the researcher to reproducibly pattern the relative abundance of 120 human serum proteins between two samples, such as serum samples from diseased and healthy individuals.



Serum Biomarker Chip Kit

The Serum Biomarker Chip Kit includes two arrayed dual-pad slides, two incubation chambers and Whatman protein array wash and blocking buffers. The slide holder and labeling/detection reagents are available separately. The chip can be scanned with any standard fluorescence scanner.

Alpha fetoprotein	Haptoglobulin	MMP-2	
Alpha 1 antichymotrypsin	Hemoglobin	MMP-3	
Alpha 2 macroglobulin	Hepatocyte growth factor	MMP-9	
Angiogenin	ICAM-1	Myeloperoxidase	
Angiopoietin-2	IgA	Myoglobin	
Angiostatin	IgG	Neuron-specific enolase	<del>)</del>
Apolipoprotein	IgM	RANTES	
Apolipoprotein J	IL-1α	Osteopontin	
Beta-2 microglobulin	IL-1β	PDGF (all isoforms)	
Bone sialoprotein	IL-2	PDGF (BB isoform only)	
CA 15-3	IL-2 receptorα	Placental alkaline phosp	ohatase
CA 19-9	IL-2 receptorβ	Plasminogen	
CA 50	IL-3	Plasminogen activator i	nhibitor
CA 125	IL-4	Prostatic acid phosphat	ase
Carcinoembryonic antigen (group 2 specific)	IL-5	PSA (free)	
Carcinoembryonic antigen (group 4 specific)	IL-6	PSA (total)	
Cathepsin B	IL-7	PSA-ACT complex	
Ceruloplasmin	IL-8	S100	contd

46 FAST Macro Serum Biomarker Chip 47

Chondroitin sulfate	IL-10	Serum albumin
Chorionic gonadotropinα	IL-12p40	Sialyl Lewis X
Chorionic gonadotropinβ	IL12-p70	TAG-72
Chromagranin	IL-13	Tetranectin
Collagen type I	IL-17	TGFα
Complement C4	Insulin	TGFβ
C-reactive protein	Insulin growth factor binding protein 3	Thrombospondin-1
Cyclin-dependent kinase inhibitor 2A	Insulin-like growth factor 1	Thrombopoietin
Cytokeratin fragment 21-1 (CYFRA 21-1)	Interferonγ	Thyroglobulin
Eotaxin	IP-10	TIMP1
Epidermal growth factor	Kallikrein-5	TIMP2
Epidermal growth factor receptor	Kallikrein-9	ΤΝΓα
ErbB2	Kallikrein-12	TNFβ
E-selectin	Kallikrein-14	Transferrin
Estrogen receptor	Laminin	Tumor-associated trypsin inhibitor
Fas	Low-density lipoprotein	Tyrosinase
Fas ligand	MCP-1	Urokinase plasminogen activator
Ferritin	MCP-2	VCAM-1
Fibroblast growth factor-7	MCP-3	VE-cadherin
Fibroblast growth factor-basic	MCP-4	VEGF
G-CSF	M-CSF	VEGF-D
GM-CSF	MIP-1α	Von Willebrand factor

Ordering Information - Serum Biomarker Chip			
Description	Quantity/Pack	Catalog Number	
Serum Biomarker Chip Kit	1	10 486 077	
Each Kit includes:			
Serum Biomarker Chip Arrayed FAST Slides	2		
Dual-Pad Incubation/processing Chambers	2		
Protein Array Wash Buffer	1 x 125 mL		
Protein Array Blocking Buffer	1 x 5 mL		
Fast Frame Slide Holder - for 4 slides	1	10 486 001	
Chip Clip Slide Holder for 1 slide	1	10 486 081	

The Serum Biomarker Chip is intended for research purposes only, not for diagnostic use

# Two-Color Labeling and Detection System

The Whatman Two-Color Labeling and Fluorescent Detection Kit is designed to label two protein samples. The labeled proteins are pooled and probed against arrayed antibodies in a competitive binding assay, and detected using indirect fluorescence.

The kit is intended for use with the 2-pad FAST Slides, including the Serum Biomarker Chip. The kit contains the Universal Linkage System (ULS) chemistry to label samples containing approximately 250 µg of protein in serum, plasma or a whole cell lysate. The kit is designed to label two different protein samples, each with a different hapten. Sufficient labeling reagent is provided to perform a hapten swapping experiment.

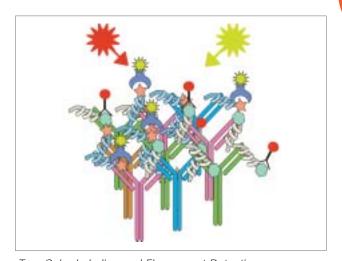
#### Features

- Highly efficient and uniform labeling of complex serum samples
- Reproducible labeling and signal detection
- Stable, robust and fast non-enzymatic procedure
- Reduces pH dependency of labeling efficiency
- System solution includes labeling reagents, fluorescent conjugate and bench-friendly protocol
- Accounts for hapten-specific differences in either Biotin-ULS or Fluorescein-ULS labeling efficiencies
- Averages differences in antibody-antigen binding interactions caused by steric hindrance
- Minimizes chip-to-chip variability includes an internal control within the assay

The first pad on the slide is probed with a mixture of two different protein samples, each labeled with a different hapten; the second pad is probed with the same two protein samples but with the haptens reversed. The normalized intensity for each element of each pad is calculated as the average of the biotin- and fluorescein-labeled derived intensities from a two-pad experiment. The ratio between the signal intensity at each spot corresponds to the concentration ratio of the proteins found in the two samples. This method is attractive for antibody chips as it takes into account any hapten-specific differences in antigen-antibody interactions.



Two-Color Labeling and Fluorescent Detection Kit



Two-Color Labeling and Fluorescent Detection

Two-Color Labeling and Detection System 49

The use of the ULS labeling system minimizes background by using indirect fluorescence detection, labels multiple amino acids and requires no additional materials or reagents.

Ordering Information - Two-Color Labeling and Detection Kit				
Description	Quantity/Pack	Catalog Number		
Two-Color Labeling and Fluorescent Detection Kit	1	10 486 085		
Each kit includes:				
Biotin-ULS	20 μL			
Fluorescein-ULS	20 μL			
10x Protein Labeling Buffer	80 μL			
10x KREAstop	80 μL			
Streptavidin-DY 647 Conjugate	150 μL			
Anti-Fluorescein Antibody-DY 547 Conjugate	350 μL			
Micro Bio-Spin Chromatography Columns	8			
User's Manual	1			

The Whatman Serum Biomarker Chip is intended for research purposes only, not for diagnostic use

# ArrayVision® FAST®

#### Data Analysis Software

ArrayVision is a flexible software package that has been developed for the quantification of gene expression arrays. It is perfectly suited for the quantification of proteins.

It provides rapid and automated analysis for array images in a few easy steps. The software is designed with maximum flexibility to suit the needs of any size lab and uses configurable protocols to suit any array format.



#### ArrayVision FAST for use with FAST Quant

#### Features

- · Quantitative analysis of protein arrays
- Template definitions and analysis parameters
- · Accepts .tif images from any fluorescent scanner
- Interpolates the concentration for each analyte offered in FAST Quant
- · Standard curve generation through non-linear curve fitting

- Determination of cytokine concentration in unknown samples
- · Coefficient of variation calculations
- Flexible data export to most data mining packages
- Multiple curve equations for each analyte

When ArrayVision is used with FAST Quant, data can be analyzed within minutes after scanning to provide quantitative results. ArrayVision FAST includes templates designed to fit FAST Quant and can also be used to analyze scanned Serum Biomarker Chip slides.

### ArrayVision FAST - Data Analysis Software

Calculation of density values (signal intensity) for each spot on the array, combined with standard curve generation and concentration determination

Analysis of .tif images from microarray scanners

Multiple linear or non-linear curve fitting algorithms

Artifact removal and data flagging

Compatible with all major brand scanners

Supported export formats: XLS, CSV, WKS, TXT, PRN

Ordering Information - ArrayVision FAST - Data Analysis Software				
Description	Catalog Number			
Single user software	10 486 035			
ArravVision Demo USA/Canada	10 486 034			

# Protein Array Services

Whatman offers a comprehensive group of protein array services, ranging from contract arraying to slide processing to scanning and data analysis, all based on the widely accepted FAST Slide protein microarray platform.

Whatman recognizes that not all scientists have access to the instrumentation or software needed to process, image and analyze microarray data. In addition, unfamiliar microarray techniques and processing protocols may burden researchers with unforeseen obstacles or discourage researchers from adopting a new technology.



Array Room

50 ArrayVision FAST Protein Array Services 51

Whatman protein array services allow the researcher to focus on the elucidation of data and the development of subsequent studies, while Whatman delivers reliable data and images.

At our protein array facility in Sanford, ME, USA, Whatman can array proteins in Class 10,000 clean room using solid pins, split pins or non-contact piezoelectric pins.

If you choose from our list of available antibodies or if you send us your own content, we can design an array, print and have slides to you within 15-20 business days of your order.

## Antibody Menu

Whatman offers custom sample processing using an extensive antibody menu.

Whatman	Protein Ar	ray Service	es - Antibo	ody Menu			
Human	Sensitivity	Dynamic	Dose	Mouse	Sensitivity	Dynamic	Dose
Cytokines	(pg/mL)	Range	Response	Cytokines	(pg/mL)	Range	Response
Available		(pg/mL)	Slope	Available		(pg/mL)	Slope
IL-1β	3	3-1,000	0.82	IL-1β	3	3-1,000	0.73
IL-2	3	10-1,000	0.88	IL-2	3	3-1,000	0.82
IL-4	3	10-3,000	0.84	IL-4	3	3-1,000	0.83
IL-5	10	10-3,000	1.11	IL-5	3	3-300	1.6
IL-6	3	10-3,000	0.87	IL-6	3	3-1,000	0.8
IL-8	3	3-3,000	1.1	IL-10	3.2	24-20,000	1.2
IL-10	30	100-3,000	0.68	IL-12p40	3.2	3.2-400	0.76
IL-12p40	30	30-10,000	0.85	IL-12p70	3.2	3.2-400	1.1
IL-12p70	30	100-10,000	1	IL-13	3.2	24-20,000	1.1
IL-13	100	100-3,000	0.69	TNFα	7	7-1,000	0.77
IL-17	3	3-1,000	0.7	IFNγ	3.2	6-400	1.3
IL-10	30	30-1,000	0.55	VEGF	3.2	32-2,000	1.1
ICAM-1	100	100-3,000	0.97	RANTES	10	10-1,000	0.74
TNFRII	50	100-30,000	0.63	MIP-1α	10	30-1,000	1.2
TNFα	3	3-1,000	0.93	MIP-2	1	3-1,000	0.93
IFNγ	10	10-3,000	0.58	GM-CSF	1	1-300	0.81
Angiogenin	30	30-1,000	0.79	M-CSF	3	3-1,000	0.82
VEGF	50	100-30,000	0.96	MCP-5	3	3-300	0.81
TGFβ	30	100-10,000	0.93	IL-3	3	3-1,000	0.90
RANTES	3.2	3.2-400	0.9	-	-	-	-
MIP-1α	16	16-400	0.76	-	-	-	-
GM-CSF	1	1-400	0.78	-	-	-	-
M-CSF	3.2	3.2-400	0.75	_	-	_	- contd

Human	Sensitivity	Dynamic	Dose	Mouse	Sensitivity	Dynamic	Dose
Cytokines	(pg/mL)	Range	Response	Cytokines	(pg/mL)	Range	Response
Available		(pg/mL)	Slope	Available		(pg/mL)	Slope
MCP-1	1	3.2-400	0.9	-	-	-	-
MCP-2	1	1-300	0.74	-	-	-	-
MCP-3	1	1-300	0.87	-	-	-	-
MCP-4	3	3-300	0.88	-	-	-	-
EGF	1	1-30	0.87	-	-	-	-
IGF-1	10	10-1,000	0.62	-	-	-	-
IL6-R	1	1-1,000	0.77	-	-	-	-
Angiopoietin-2	3	3-300	0.31	-	-	-	-
Eotaxin	3	3-300	0.69	-	-	-	-
FGF-basic	10	10-1,000	0.82	-	-	-	-
IL-1α	1	1-300	0.84	-	-	-	-
IL-3	3	3-300	0.79	-	-	-	-
IL-7	1	1-100	0.92	-	-	-	-
KGF	10	10-1,000	0.76	-	-	-	-
PDGF-BB	10	10-300	1.0	-	-	-	-
TIMP-1	3	3-1,000	0.88	-	-	-	-
Тро	30	30-1,000	1.5	-	-	-	-

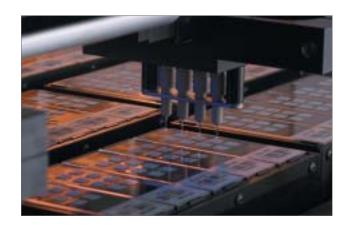
Note: Whatman is adding to its antibody menu on a continual basis. Please inquire regarding the latest additions.

## Developing Custom Arrays

### Creating an Antibody-based Protein Microarray

The custom array service at Whatman enables scientists who have access to microarray scanning and data analysis instrumentation to have FAST Slides printed with proteins from the Whatman antibody menu or from their own protein library.

The custom service provides the scientist complete control of proof-of-principle, assay design, processing and data analysis of the printed array.



52 Protein Array Services 53
Protein Array Services

Customer-supplied biological samples are screened against the selected specificities determining the quantitative or qualitative measurements, depending upon the customer's requirements. The deliverables to the researcher include access to raw and trimmed data, total fluorescent signal per microspot (quantitative assays only), % CV and standard deviation, signal-background per microspot intensities versus (y-axis), each analyte in the sample (x-axis), and graphed histogram of ratios, signal-background averages, and outlier recognition.

Protein array development projects are driven by certified R&D project leaders responsible for the scope, resources and timeline that meet the needs of the researcher. Project implementation, project milestones and customer communications are managed by dedicated account specialists, in collaboration with the research staff.

Protein specificities, whether they are from Whatman or customer supplied, are arrayed to the selected FAST Slide configuration - using a quill-pin contact or non-contact printing technology - in triplicate, unless otherwise specified - suspended in Whatman protein arraying buffer to achieve long-term stability.



Microspot ELISA and Antibody Arrays

#### Creating an Antibody-based Protein Microarray

**Step 1:** Are you going to print the array yourself? If yes, look to our line of FAST Slide products and array buffers to ensure outstanding results.

**Step 2**: Would you like to array from our available menu? If yes, simply contact us with which specificities and arrays you would like. Our experts will define the best adapted configuration to your specific arrays. Our menu is constantly expanding and we are happy to accommodate specific requests. Select the analytes you need to have printed from the antibody menu or send your own library of proteins. Receive your custom arrayed FAST Slides within 15-20 business days.

**Step 3**: Would you like for us to process your samples on a custom or off-the-shelf array? If yes, simply contact us and provide number and type of samples.

## Processing Arrays

Using leading-edge, automated microarray technologies and innovative scientific approaches, the protein array development, processing and data analysis service allows researchers to acquire distinct, reliable scientific data from the proteomic specialists at Whatman. Protein array development, within our facility, encompasses proof-of-principle, assay design, array design and printing, processing and data analysis.



#### Quantitative Cytokine Array Processing and Data Analysis

Based upon the FAST Quant System, the quantitative cytokine array processing and data analysis service offers researchers the results from the quantitative analysis of multiple cytokines processed in a FAST Slide based, micro ELISA format. Signal output from FAST Quant arrays is detected with a standard fluorescent microarray scanner and data is analyzed using the ArrayVision FAST software.

- Select from the Whatman antibody menu of 40 human and 19 murine specificities
- Preserve valuable samples high throughput ELISA format requires as little as 140  $\mu$ L of human sample per array
- Applicable sample types include serum, plasma, cell culture supernatants, cellular extracts and wound effluent
- Reduces costs associated with the cost of antibodies, capital equipment, assay development, lab personnel software acquisition and analysis
- Processed and analyzed by expert staff in dedicated array processing facility

The following FAST Quant data and images are uploaded to the FTP site:

- Total fluorescent signal per microspot
- Standard curves per cytokine
- % CV
- Standard deviation
- pg/mL value per sample

54 Protein Array Services 55

#### Comparative Analysis of Known Serum Biomarkers

The Serum Biomarker sample processing service makes it simple for researchers to outsource the tests or try this new technology before adopting it in their labs.

- Customer samples sent to Whatman and processed using the Serum Biomarker Chip
- · Data uploaded to a password-protected FTP site within ten business days of receipt of samples at Whatman
- Minimum order: two paired serum samples

The following Serum Biomarker Chip processing data and images are uploaded to the FTP site:

- Total fluorescent signal per microspot
- % CV
- Standard deviation
- Signal-background per microspot intensities versus (y-axis)
- Each analyte in the sample (x-axis)
- Graphed histogram of ratios
- Average of signal-background triplicate microspots
- · Z score to identify outlier microspots that are significantly different from the mean

## Scanning Arrays

Whatman offers a slide scanning and data analysis service for FAST Slide users who do not have access to a fluorescent scanner.

The scanning service is limited to arrayed and processed FAST slides, processed FAST Quant slides and processed Serum Biomarker Chip slides.

Using the GenePix 4200 A Professional Microarray Scanner, FAST Slides or FAST array products can be simultaneously scanned at two wavelengths; the resulting .bmp, .tif or .jpg file formats can be easily downloaded from the Whatman password protected FTP site.

Upon ordering the slide scanning and data analysis service, the customer will receive the scanning service pack, including shipping container(s) designed to transport the slides safely, a prepaid FedEx clinical pack for shipping the slides and instructions for returning the processed slides to the array facility. Within 72 hours of receipt, the slides are scanned and images are uploaded to the FTP site.

#### Compatible Scanners

Any fluorescent scanner, either laser-based or major brand charge coupled device - is compatible with FAST Quant and ArrayVision FAST, as long as the following requirements are met: \*Resolution: 10 microns \*Capability: 1 or 2 colors \*Image output: .tif format

Ordering Information - Scanning Arrays	
Description	Catalog Number
Scanning service - 4 slides	10 486 047
Scanning service - 8 slides	10 486 049

## Slide Holders

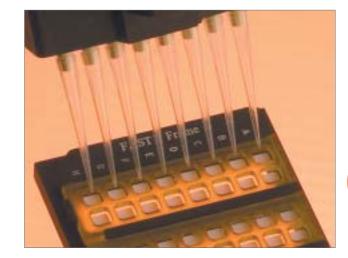
#### FAST Frame Multi-Slide Plate

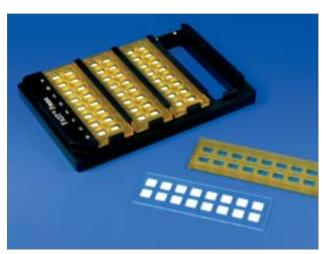
The FAST Frame multi-slide plate is designed to hold four, 16-pad FAST Slides and the corresponding multi-well incubation chambers in a microplate footprint for high-throughput processing of microarrays. The footprint dimensions meet the standards recommended by the Society for Biomolecular Screening.

The 96 Well spacing (9 mm center to center) of the array pads on FAST Slides makes the loaded FAST Frame compatible with automated liquid handling systems and 8-channel manual pipettors.

Each plate processes up to 64 arrays simultaneously. The rows and columns on each plate are labeled for easy indexing and sample application.

The FAST Frame multi-slide plate is constructed of autoclavable plastic and is compatible with standard 1" x 3" glass slides when used with Whatman reusable silicone chambers (1, 2, 16). The FAST Frame is available as a stand-alone, reusable unit or as a starter kit containing 16-pad FAST Slides, incubation chambers and chamber covers.





FAST Frame

56 Protein Array Services Slide Holders 57

<sup>\*</sup> See Specificities list in Serum Biomarker Chip section

### Chip Clip™

#### The Single Grip Slide Holder

The Chip Clip securely holds one FAST Slide and incubation chamber for processing multiple arrays simultaneously (includes the Serum Biomarker Chip). Used in conjunction with silicone incubation chambers, the Chip Clip ensures leak-proof barriers around the arrayed pads on the slide.

The slide and incubation chamber are easily inserted into and removed from the Chip Clip slide holder; side rails hold the chamber firmly against the slide surface. The Chip Clip is compatible with 25 x 76 mm (1" x 3") slides when used with incubation chambers.



Chip Clip

#### Product Specifications - FAST Frame Multi-Slide Plate FAST Frame Multi-Slide Plate Number of Slides: up to 4 9 mm Row Spacing: Footprint: 128 mm x 86 mm Material: Delrin, autoclavable Chip Clip Number of Slides: Footprint: 50 mm x 85 mm Material: Delrin, autoclavable

Ordering Information - Slide Holders					
Description	Quantity/Pack	Catalog Number			
FAST Frame Multi-Slide Plate	FAST Frame Multi-Slide Plate				
FAST Frame	1	10 486 001			
FAST Frame Starter Kit includes:	1	10 486 003			
FAST Frame	1				
- 16-pad FAST Slides	10				
- 16 Well Incubation Chambers	10				
Chamber Covers	40				
Chip Clip					
Chip Clip Slide Holder	1	10 486 081			

## Slide Incubation Chambers

Whatman array incubation chambers are ideal for protein microarray applications on FAST Slides. The chambers provide a convenient way to carry out binding reactions on protein microarrays.

Used in conjunction with the FAST Frame or Chip Clip, the incubation chambers have a secure gasket design forming a tight, leak-proof seal with the FAST Slides. Simply remove the incubation chamber when the reaction is finished. The chambers are recommended for use at room temperature and elevated incubation temperatures up to  $76^{\circ}$  C.



<b>Product Specifications - Slide Incubation</b>	Chambers
Single-Well Array Incubation Chamber*	
External dimension:	79 x 25.4 mm
Well dimension:	53 x 22 x 0.4 mm (LxWxD)
Volume:	600 - 700 μL
Dual-Well Array Incubation Chamber**	
External dimension:	79 x 25.4 mm
Well dimension:	21 x 21 x 4 mm (LxWxD)
Volume:	300 - 400 μL
16 Well Array Incubation Chamber***	
External dimension:	75 x 25.4 mm
Well dimension:	7 x 7 x 4 mm (LxWxD)
Volume:	60 - 100 μL

- \* For use with 2-pad FAST Slides, catalog 10 484 182
- \*\* For use with 2-pad FAST Slides, catalog 10 485 317

58 Slide Holders

<sup>\*\*\*</sup> For use with 8- and 16-pad FAST Slides, catalog numbers 10 485 320 and 10 485 323

Ordering Information - Slide Incubation Chambers		
Description	Quantity/Pack	Catalog Number
Single-Well Array Incubation Chamber (for 1-pad FAST Slides)	10	10 486 137
Dual-Well Array Incubation Chamber (for 2-pad FAST Slides)	10	10 486 087
16 Well Array Incubation Chamber* (for 8- and 16-pad FAST Slides)	10	10 486 046
Chamber Cover for all Chambers	40	10 485 336
FAST Frame Slide Holder, for simultaneous processing of four slides	1	10 486 001
Chip Clip Slide Holder, for single slide processing	1	10 486 081

<sup>\*</sup>The 16-Pad Incubation Chamber is used with both 8- and 16-pad FAST Slides

## Protein Array Buffers and Reagents

Whatman protein array reagents have been optimized for use on FAST Slides. These reagents include protein arraying buffer, protein array blocking buffer and protein array wash buffer.

#### **Protein Arraying Buffer**

- Enhances long-term protein stability and molecular recognition activity
- Enhances fluorescent signal from arrayed sample
- Optimized for use on FAST Slides

#### Protein Array Blocking Buffer

- Demonstrates highly efficient blocking of protein microarrays
- Exhibits strong reduction of non-specific antibody-antibody interactions
- Exhibits minimal effects on specific antibody-antigen interactions
- Results in superior signal-to-noise ratios in protein microarray applications
- Superior blocking capabilities



Protein Array Buffers and Reagents

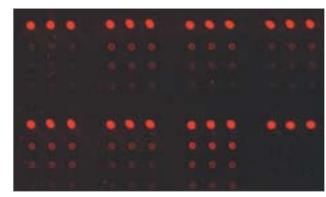
Reduction of non-specific protein-protein interaction:
Monoclonal capture antibodies were arrayed on FAST
Slides and probed with an antibody cocktail consisting
of 16 biotinylated polyclonal antibodies followed by
Streptavidin-Cy5 detection. Images were taken at
identical laser/PMT setting. The first row of the array
field is a positive detection control.

Blocking with Protein Array Blocking Buffer: Reduction of unspecific antibody-antibody interactions by the protein array blocking buffer.

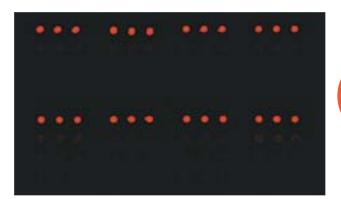
#### Protein Array Wash Buffer

- Ideal washing buffer for protein microarrays
- Conveniently supplied as 10x concentrate
- Optimized for use on FAST Slide

The Protein Array Wash Buffer has been optimized for efficient washing of protein microarrays to ensure optimum results. This buffer is used as the washing buffer in FAST PAK, FAST Quant and the Serum Biomarker Chip kits.



Blocking with TBS Tween 20, 0.1% - Unspecific Binding of Biotinylated Antibodies



Blocking with Protein Array Blocking Buffer

Ordering Information - Protein A	Array Buffers and Reage	nts
Description	Quantity/Pack	Catalog Number
Protein Arraying Buffer (2x)	4 x 10 mL	10 485 331
Protein Arraying Blocking Buffer (1x)	1 x 100 mL	10 485 356
Protein Array Wash Buffer (10x)	4 x 125 mL	10 485 330

60 Protein Array Reagents 61

# MicroCaster<sup>™</sup> Arrayer

### Handheld Microarraying System

The MicroCaster is an economical, entry-level manual microarraying system for principle and pilot studies. With the MicroCaster 8-Pin hand tool, samples can be loaded from 96 Well or 384 Well microtiter plates.

The MicroCaster slide holder holds two slides. It has a built-in indexing system that enables precise printing of up to 768 spots in an array of 32 x 24 spots. It is very easy to set up and use with processing time of 5-20 minutes per slide.

The MicroCaster is designed for 1-Pad FAST Slides with 20 mm x 51 mm pad size and is compatible with other slide surfaces.



MicroCaster Arrayer

Product Specifications - MicroCaster Arrayer			
Number of Spots:	up to 768		
Horizontal Pitch:	(x-axis): 1250 μm		
Vertical Pitch:	(y-axis): 750 μm		
Spot Size:	500 - 1000 μm		
Print Volume: (varies with buffer and viscosity)	20 - 70 nL		

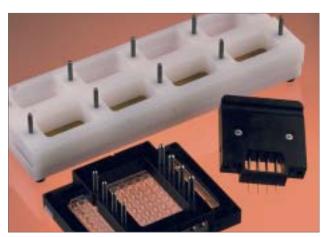
Ordering Information - MicroCaster Arrayer			
Description	Quantity/Pack	Catalog Number	
MicroCaster System*	1	10 485 047	
MicroCaster Pin Conditioner	100 mL	10 485 061	
MicroCaster Replacement Pin	1	10 485 326	
MicroCaster Pad (pin support pad)	1	10 485 370	

<sup>\*</sup> MicroCaster System includes: MicroCaster 8-Pin System Hand Tool, MicroCaster 8-Pin System Slide Holder, MicroCaster Pin Conditioner, Spare Replicator Pins

## MicroCaster Accessories

The MicroCaster accessories can be used to increase the flexibility of the manual arrayer system by providing accurate source-plate indexing and reliable pin-tool cleaning.

The MicroCaster accessories are compatible with standard 96 Well microplates and they reduce hassle with pin-tool cleaning.



Ordering Information - MicroCaster Accessories					
Description	Dimensions (mm)	Quantity/Pack	Catalog Number		
int Free Blotting Paper	80 x 115	10	10 486 042		
Wash and Blot Station	-	1	10 486 043		
96 Well Microplate Indexer	-	1	10 486 044		

62 MicroCaster Arrayer MicroCaster Arrayer 63