

KAM-850 Kit

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KINEX™ KAM-850

ANTIBODY MICROARRAY KIT CUSTOMER INFORMATION PACKAGE

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OVERVIEW OF KINEX KAM-850 ANTIBODY MICROARRAY KIT



Figure 1. Kinex™ KAM-850 Antibody Microarray Kit

1. INTRODUCTION

The Kinex™ KAM-850 Antibody Microarray Kit provides an alternative to our Kinex™ Services for clients to perform screening of their cell and tissue lysates in their own laboratories with our latest high content antibody microarray that features >850 pan- and phosphosite-specific antibodies. While our Kinex™ signal transduction protein profiling services are convenient and very cost-effective solutions to assist scientists in the broad discovery of productive research leads such as biomarkers, it can be cost prohibitive to ship frozen samples to Kinexus from overseas. The availability of the KAM-850 Kit allows clients access to our unique antibody microarrays to track the differential binding of dye-labeled proteins in lysates prepared from cells and tissues including cells, fresh or frozen tissues and serum samples. The results can provide novel and useful insights into differences in protein expression, phosphorylation and protein-protein interactions, and define antibody reagents that can be used to follow up on these findings. Our integrated platform of well established proteomics and bioinformatics services and proprietary technologies make the Kinex™ KAM-850 Antibody Microarray superior to any other commercially available antibody microarrays. Some of the advantages of our antibody microarrays include highly validated and well characterized antibody probes, wide coverage of cell signaling proteins and pathways, proprietary methods to reduce the rate of false positives and improve sensitivity, extensive follow up services for validation, and complimentary bioinformatics analyses for comparison purposes. In the following sections, we explain how the KAM-850 Antibody Microarray performs and how it stacks up to the competition. In Appendix B, we provide a direct comparison with high content antibody microarrays available from other competitors.

2. HIGHLY VALIDATED ANTIBODIES

Our current full Kinex™ Antibody Microarray Service monitors changes in the expression levels and phosphorylation states of signalling proteins with more than 850 antibodies, which includes approximately 517 pan-specific antibodies (for protein expression) and 337 phospho-site-specific antibodies (for phosphorylation). These antibodies, which have been selected from more than 4600 different commercial antibodies from over 26 companies, have been independently tested by Kinexus to identify many of the best immunological reagents available today to track important signal transduction proteins. Nearly 75% of the tested antibodies failed our tests for potency and specificity. The other top 25% of these antibodies that performed well in Western blotting applications have been incorporated into our Kinex™ Antibody Microarrays. Such cherry-picking is apparently not performed by our competitors, which rely only on one or two suppliers with dubious information about individual antibody performance. When our clients utilize the KAM-850 antibody microarray, upon request, we are pleased to disclose the commercial sources and in some cases, these antibodies are available from Kinexus for as low as US\$179. Immunoblots with these particular antibodies are available for easy viewing in "Our Products" section of our main website. A complete listing of all the antibodies printed on the KAM-850 chip is shown in Appendix C, and an MS-Excel file with these antibodies is downloadable from the Kinexus website and provided on a DVD included with the KAM-850 Antibody Microarray Kit.

The classes of targeted proteins and phosphosites with the antibodies on the KAM-850 Antibody Microarray are listed in Table 1 below. The antibodies selected for use in our microarrays have been optimized to work in human, mouse and rat model systems, but have also been shown commonly to work in chicken, bovine, porcine, canine, rabbit, frog, sea star and many other diverse model systems.

Table 1. Families of protein targets for the KAM-850 Antibody Microarray chip. These statistics apply to Lot: K00114 (October 2012) and may be slightly altered in future print runs of this microarray chip. "Redundant" antibodies are those extra antibodies that react with different epitopes on the same protein targets as the "Non-redundant" antibodies or often with multi-phosphorylated phosphorylation site sequences. The other antibodies cover a range of other cellular functions, including cell cycle and apoptosis.

Content	Total	Non-redundant	Redundant
Number of pan-specific antibodies: 517	60.5%		
Number of phospho-specific antibodies: 337	39.5%		
Number of protein kinase antibodies - pan-specific	309	189	120
Number of protein kinase antibodies - phosphosite-specific	157	128	29
Number of protein phosphatase antibodies - pan-specific	38	31	7
Number of protein phosphatase antibodies - phosphosite-specific	6	4	2
Number of stress antibodies - pan-specific	37	22	15
Number of stress antibodies - phosphosite-specific	9	6	3
Number of transcription antibodies - pan-specific	24	20	4
Number of transcription antibodies - phosphosite-specific	51	44	7
Number of other antibodies - pan-specific	109	100	9
Number of other antibodies - phosphosite-specific	114	105	9
Total Number of Antibodies	854	649	205

3. QUALITY CONTROL PROCEDURES

Our antibodies are covalently immobilized on high quality glass surface coated with a proprietary 3-D polymer material to ensure high binding efficiency and specificity. Our microarrays are subjected to stringent quality control measures designed to ensure optimum antibody binding activity, printing consistency, and consistent intra-slide and inter-slide variability. Each microarray also has loading and antibody controls to ensure the distribution of protein is consistent on all fields. The KAM-850 Antibody Microarray chip provides semi-quantitative analyses of the expression and phosphorylation states of cell signalling proteins in two cell and tissue samples. If clients wish to take advantage of our KAM-850 Report Service, quantitative analysis of the strength of the fluorescence signals for each target protein can be provided in duplicate in a Microsoft Excel spreadsheet, which includes the (average) percent change from the control sample, the percent range in error, and Z-ratios.

In internal studies with the latest KAM-850 Antibody Microarray chip, we determined that the median spread between duplicate measurements with the same antibody in printed pairs was about 24% (i.e. the median range from the average of the duplicates was $\pm 11.9\%$ with a standard deviation of 1.0% from testing of 10 fields of 854 antibody pairs per field). The frequency of flagged antibody spots due to dust or mis-printing is less than 0.5%. When the average of duplicate measurements of antibody pairs on each chip was determined for the same sample applied to different KAM-850 Antibody Microarrays, we observed that the median value for the differences in the averages was $\pm 8.1\%$ with a standard deviation of 0.6% from testing of 4 pairs of fields. The dynamic range between the highest and lowest reproducible dye-bound protein signals from these Kinex™ chips exceeded 5000-fold. This performance exceeded that of antibody microarrays from three other competitors tested in our hands.

Figures 2 and 3 below provide images of the Kinex™ KAM-850 Antibody Microarray following a scan for dye-labeled protein from lysates from control and treated cells. To view additional example images or a sample of a Kinex™ KAM-850 Report, please contact one of our Technical Service Representatives.

Figure 2. Scanned image of the entire Kinex™ KAM-850 Antibody Microarray.

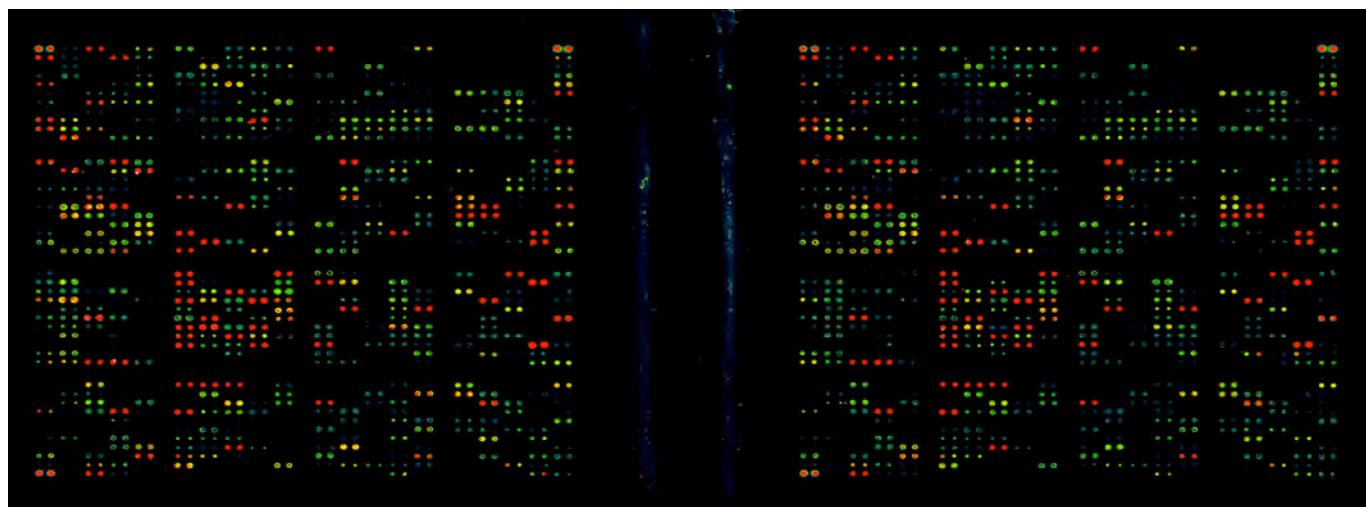
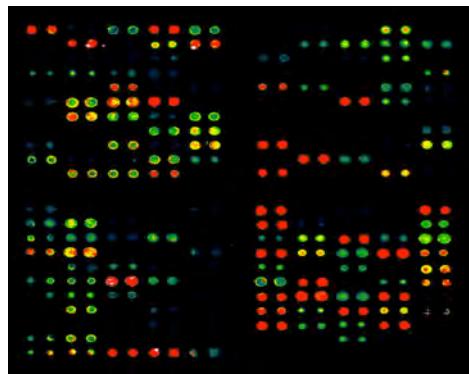


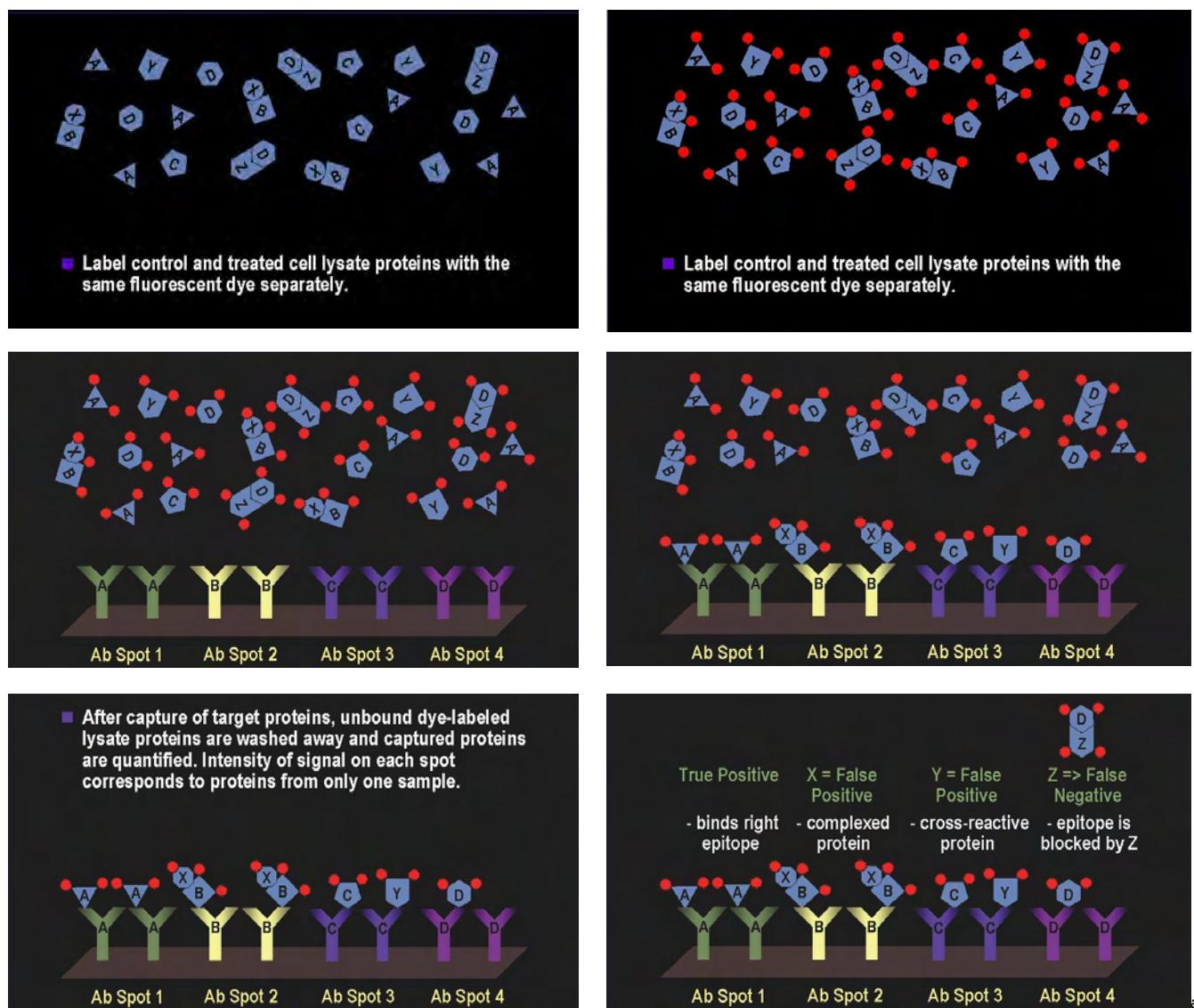
Figure 3. Close up of 4 out of 32 grids on the Kinex™ KAM-850 Antibody Microarray.



4. PRINCIPLES OF BINDING AND DETECTION

The methodology behind the Kinex™ KAM-850 Antibody Microarray is illustrated in Figure 4. The issues of antibody cross-reactivity, protein complexes and epitope masking are highlighted in last panel.

Figure 4. Methodology used in Kinex™ KAM-850 Antibody Microarray.



5. PROPRIETARY DYE

One key advantage of our antibody microarray format is that lysate samples from control and treated cells are labeled with the same dye and analyzed together on the same chip at the same time. In our experience, the use of a two dye, competitive binding system, in which a control sample is labeled with a different dye from the treatment sample and the two samples are mixed and co-incubated with the same regions of the same chips is not advisable. Apart from a 50% reduction in the amount of maximum detectable signal, it generates a much higher rate of false leads. Unlike oligonucleotides such as DNA and RNA, proteins display strong individual differences in their relative affinities for dyes. It should be appreciated that this problem also significantly impacts other proteomics approaches such as DIGE 2D gel analysis where two samples that are labeled with different dyes are mixed prior to electrophoresis. Colour changes seen with spots evident on a DIGE 2D gel may not be related to differences in protein expression but rather to variations in dye binding to individual protein species. Clients should also be aware that cell signalling proteins are typically present at concentrations that are 100- to 1000-fold lower than structural proteins and metabolic pathway enzymes. Consequently, these low abundance proteins are usually not evident on 2D gels without some type of special pre-enrichment. This is why we feel that antibody-based detection of proteins with our Kinex™ KAM-850 Antibody Microarrays and follow up Kinetworks™ Custom Screens are superior and complementary methods to undertake broad studies of proteins for signalling network analyses.

6. CHEMICAL CLEAVAGE

Since non-denatured proteins are analyzed by this method, as illustrated in the last panel of Figure 4, there is increased opportunity for false positives and false negatives due to antibody cross-reactivity and blocked epitopes in protein complexes. Many proteins reside in complexes with other proteins and antibodies, and as it is normally necessary to use non-denaturing conditions with antibody microarrays, many apparent changes in protein expressions or phosphorylations may arise from alterations in protein-protein interactions.

In our internal studies with cells from different species, only between 30 to 45% of the protein changes detected on a protein microarray were reproduced by immunoblotting. In addition, about 15 to 20% of the protein changes could not be validated by immunoblotting, because no detectable immunoreactive proteins were evident in these studies as the antibody microarray appears to be about 10-fold or more sensitive than standard Western blotting. It should be appreciated that this high rate of false positives is an inherent problem with all commercial antibody microarrays due to the reliance on non-denaturing conditions for immune capture of target proteins. To help reduce the number of false positives that are typically generated on a protein microarray, we have provided instructions for a chemical digestion step with the KAM-850 Kit in which native proteins are cleaved into larger fragments. This fragmentation leads to dissociation of complexes, but does not destroy most of the epitopes recognized by phosphosite-antibodies. Typical enzymatic cleavage of proteins with proteases such as trypsin causes the loss of most phosphosite epitopes as basic amino acids commonly surround phosphoserine and phosphothreonine sites. This chemical digestion step as an option to reduce the number of false positives for clients that are less interested in tracking protein-protein interactions changes in experimental model systems. The reagents for this chemical cleavage step are NOT provided with the KAM-850 Kit as many users may wish to also observed changes in protein-protein interactions in lysate samples from specimens from humans and animals.

7. COMPLIMENTARY MICROARRAY SCANNING

Clients must be able to scan the KAM-850 Antibody Microarray with captured dye-bound lysate proteins to quantify the levels of these proteins and their phosphorylation status in their cell and tissue samples. If suitable microarray

scanners are not locally available, clients have the option of shipping their chips back to Kinexus for complimentary scanning. The processed KAM-850 Antibody Microarray chip should be couriered back in the same plastic array storage receptacle tube in which it was packaged in the KAM-850 Kit. Refrigeration or freezing of the chip is unnecessary for shipping. Clients must arrange and pay for the courier costs for delivery to Kinexus in Vancouver, B.C., Canada. JPEG images of the scanned chip will be returned to the client by e-mail.

8. KAM-850 ANTIBODY MICROARRAY REPORTS

If clients wish Kinexus to quantify the intensities of dye-bound proteins captured on the KAM-850 chip, we offer a supporting service in which we use our proprietary software to average the intensities recorded for each pair of antibody spots to calculate the differences between the control and treated lysate samples. This includes calculations of Z scores and percent changes from control (%CFC). This permits the identification of the most promising biomarkers for further validation by immunoblotting. The Report in pdf and MS-Excel formats is returned back to clients within a 5 day turnaround by e-mail. The cost of this service for each Report in which one control and one experimental samples are compared is US\$195. Additional costs may apply if extra comparisons are requested.

9. FOLLOW-UP SERVICES

We highly recommend that all interesting leads generated with the Kinex™ KAM-850 Antibody Microarray should be validated by Western blotting. Such validation is essential with any commercial or custom produced antibody microarray. To assist in this regard, Kinexus offers two cost-effective custom immunoblotting services. Clients can choose from the Kinetworks™ Custom KCPS 1.0 (Multi-Antibody) Protein Screen where any 18 antibodies can be selected and we will optimize it to your model system, or with the Kinetworks™ Custom KCSS 1.0 (Multi-Sample) Protein Screen send up to 8 different samples and choose up to 3 different antibodies, provided the molecular weights are significantly separated by SDS-PAGE. Lysate samples for Kinetworks™ analyses may be shipped without refrigeration to Kinexus following boiling in SDS-PAGE sample buffer. More about these Kinetworks™ services and the necessary forms can be download from our website with the following specific url: http://www.kinexus.ca/ourServices/immunoblotting/custom_profiling/custom_profiling.html

The availability of these Kinetworks™ Custom screens is an important distinguishing feature of our antibody microarray services as clients can have their research leads conveniently and cost-effectively confirmed. The cost savings arising from the use of the Kinexus discovery platform becomes immediately apparent when one considers the purchase costs of individual antibodies and the labour necessary to confirm key antibody results obtained with competitor antibody microarrays. In addition, once the results are confirmed by Western blotting, clients can correlate their data with thousands of other data points from hundreds of different model systems using our KiNET databases, which contain the results from thousands of Kinetworks™ Immunoblots or Kinex™ Antibody Microarray analyses. Over 370 scientific publications have been published that reference the Kinexus Services, of which more than 100 are directly related to the Kinex™ Antibody Microarray Service.

In addition to the Kinetworks™ Custom Immunoblotting Services to validate leads, Kinexus can assist with many other aspects of your research project from start to finish. Other services that can be used in combination with our Kinex™ Antibody Microarray services include the following:

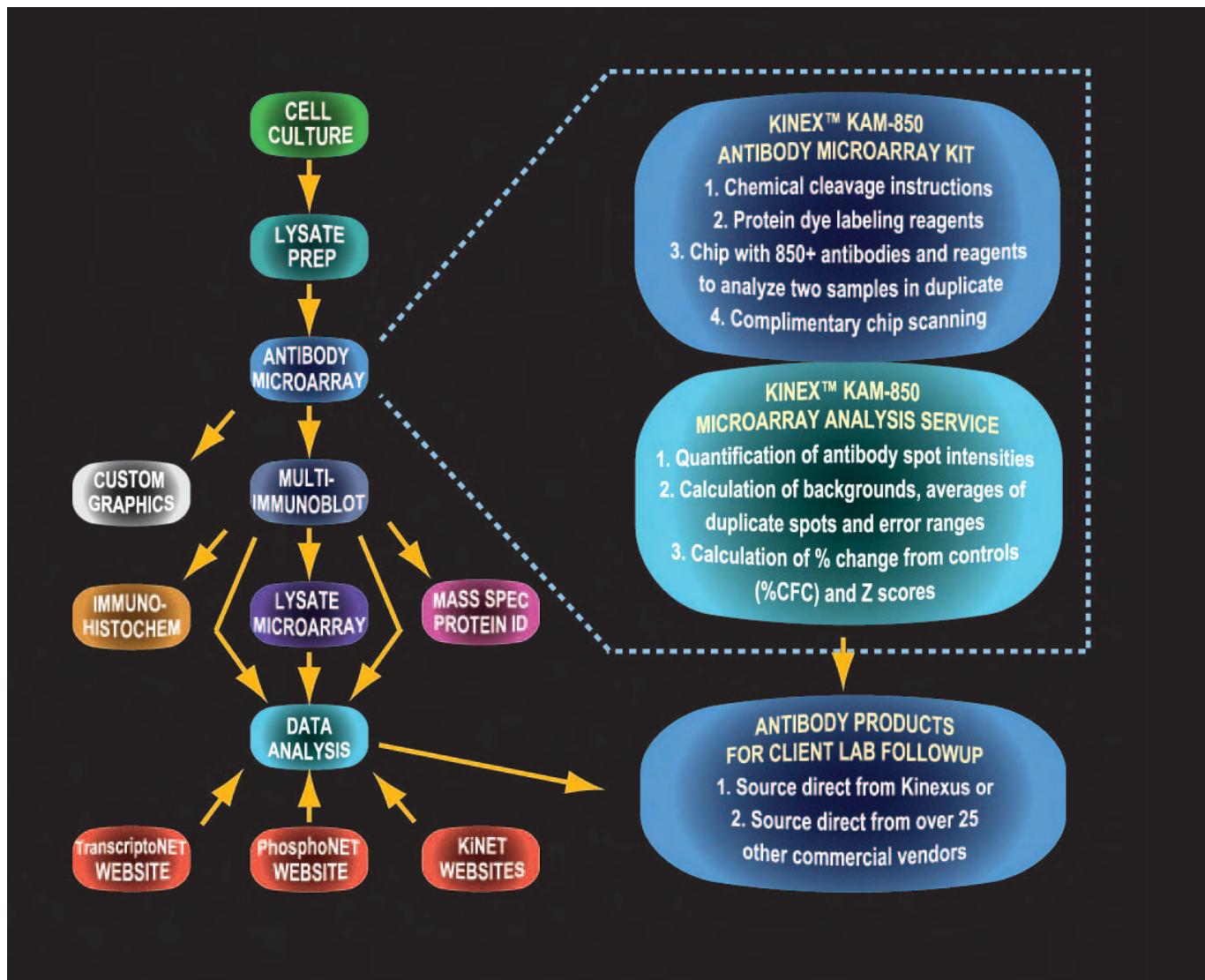
- In vivo services – send us your experimental compounds, proteins or oligonucleotides and we will perform the treatment of cells according to your specification and generate lysates for testing with our microarrays;
- Tissue or cell pellet processing – send us your cell pellets or tissues and we will prepare lysates for you;
- Concentrating – we can increase the concentration of your samples if required;
- Dye-labeling step – we can provide an extra dye-labeling step if your sample concentrations are low;
- Chemical digestion step – we can apply our proprietary cleavage step to reduce false positives;
- Mass spectrometry identification of antibody cross-reactive proteins;
- Custom graphics – we can prepare pathway charts and bar graphs for your scientific publications;
- Custom Microarrays – we can print custom antibody microarrays for your own internal research programs;
- Custom Lysate Microarrays – we can print custom microarrays with hundreds of lysates to allow for further evaluation of the biological robustness of biomarkers identified through our Kinex™ Antibody Microarray services.

The aforementioned services are available for nominal fees. However, Kinexus also offers free services and open access online databases to clients which include the following:

- Free reagents – we can send clients an aliquot of our lysis buffer and protease inhibitor tablets to assist with the sample preparation process. We require just a courier account number to cover the shipping fees;
- KiNET™ Antibody Microarray (KiNET-AM) DataBase – clients can directly compare their Kinex™ Antibody Microarray results with lysates from thousands of other experimental model systems analysed with the same methodology;
- KiNET™ Immunoblotting (KiNET-IB) DataBase – clients can compare the results from their validation immunoblotting data with hundreds of other experiments from hundreds of other model systems.
- PhosphoNET KnowledgeBase – clients can compare interesting phosphosites identified by our microarrays with over 650,000 confirmed and predicted human phosphosites to learn about their evolutionary conservation in up to 20 different species as well as the top kinases predicted to phosphorylate these sites;
- TranscriptoNET KnowledgeBase – clients can compare expression levels identified by our microarrays with the mRNA levels for over 21,000 human genes in 600 different human organs, tissues and cell lines.

Figure 5 outlines how the proteomics and bioinformatics services and products offered by Kinexus integrate into a discovery engine for identification and validation of biomarkers and antibody probes to track them with other detection systems.

Figure 5. The Kinexus integrated platform of proteomics and bioinformatics services and products for biomarker discovery and validation, and antibody probe identification. The Kinex™ KAM-850 Antibody Microarray Kit enables clients to perform initial analyses in their own laboratories, but still gain access to a wide range of follow-up services and products.



KINEX™ KAM-850 KIT COMPONENTS



Figure 6. Opened Kinex™ KAM-850 Antibody Microarray Kit.

10. LIST OF COMPONENTS AND ADDITIONAL ITEMS NEEDED

Each Kinex™ KAM-850 Antibody Microarray Kit is designed for processing and probing a pair of protein samples for comparison. It contains all the necessary components required including a KAM-850 microarray chip, reagents and consumables for protein lysate preparation, protein labeling and purification, array blocking, probing and washing. It can be shipped by courier at room temperature. We also offer the KAM-850 microarray chip and the supporting reagents and consumables as separate products. Upon arrival, remove the KAM-850 microarray in the plastic array storage receptacle tube from the kit and store it unopened at -20°C or colder until use. The Kinex 543 Dye vials should also be kept at -20°C. Store the rest of the components of the kit at 4°C, and we recommend that the kit is used within 6 months of receipt.

The KAM-850 Kit contains the following components:

- KAM-850 microarray chip – 1
- Plastic array storage receptacle tube - 1
- Kinexus Protein Lysis Buffer - 1 ml
- Protein Labeling Buffer I – 25 µl

- Protein Labeling Buffer II – 250 µl
- Kinex 543 Dye - 2 vials
- Microspin G25 Columns - 2
- Blocking Buffer I - 2 ml
- Blocking Buffer II – 500 µl
- 10X Incubation Buffer – 4 x 2 ml
- 10X Wash Buffer I - 2 ml
- 10X Wash Buffer II – 2 ml
- 10X Wash Buffer III - 2 ml
- 10X Wash Buffer IV - 2 ml
- KAM-850 2-well Incubation Chamber - 1
- Dithiothreitol powder – 1 mg
- Collection tubes – 2
- A DVD with complete instructional information for KAM-850 processing

Additional components and access to the following lab equipment is needed:

- Pipettes
- Microprobe sonicator or syringes with 26-gauge needles
- Homogenizer or French Press (for tissues)
- Roche Mini Complete Protease Tablet
- Benchtop ultracentrifuge or microcentrifuge
- Benchtop swing bucket centrifuge (optional)
- Spectrophotometer equipped with a visible light source
- Vortexer
- Aluminum foil
- Bradford Protein Assay reagents
- Benchtop orbital shaker
- Rotator
- Compressed N₂ (optional)
- Microarray scanner (e.g. ScanArray® Express Microarray Scanner from Perkin-Elmer, GenePix 400B Microarray Scanner from Molecular Devices)

11. QUANTITY OF LYSATE REQUIRED

The amount of lysate protein required for the Kinex™ Antibody Microarray analysis is 50 µg per sample at an approximate concentration of 2 mg/ml. Two lysate samples are tested on the same microarray chip. A lysate preparation buffer is provided with the Kinex™ Antibody Microarray Kit. Alternative lysate buffers may be used, but they must NOT contain Tris or reagents carrying reactive amine groups. Lysate samples should be stored frozen prior to dye labeling and application to the microarray chip.

ORDERING INFORMATION

12. FORMS TO BE COMPLETED

Only one is initially necessary and is provided with this information package in Appendix A. The Kinex™ KAM-850 Kit Product Order Form should be completed and transmitted back to Kinexus by facsimile or e-mail to place an order for the product. Additional forms are provided on a DVD that is sent with the Kinex™ KAM-850 Kit. These include a Kinex™ KAM-850 Scanning Service Order Form, which should be sent along to Kinexus with the lysate-incubated KAM-850 chip in the event that clients would like to have scanned as a complimentary service. If the KAM-850 chip is being sent to Kinexus from outside of Canada, it will be necessary to complete a commercial invoice form for passing Canadian Customs. Clients are responsible for arranging shipping of microarrays sent to Kinexus and paying the courier costs. If clients wish to have Kinexus provide a KAM-850 Analysis Report, they should fill out a KAM-850 Analysis Report Service Order Form and submit it by e-mail with a high resolution Tiff image of the scanned KAM-850 chip or by courier along with the lysate-incubated microarray.

A. Kinex™ KAM-850 Kit Product Order Form (KABM-POF)

Please ensure:

- Address and contact name and numbers are specified
- Billing or accounting information is completed
- Any quotations are listed in the billing sections
- Include a Purchase Order, Visa or MasterCard number for payment
- The form is certified correct with a signature and dated



Form: KABM-POF

**KINEX™ KAM-850
ANTIBODY MICROARRAY KIT****PRODUCT ORDER FORM****KINEXUS ORDER NUMBER**

For Kinexus internal use only.

CUSTOMER INFORMATION REPEAT CUSTOMER OR NEW CUSTOMER Dr. Mr. Ms.

Name of Authorized Representative or Principal Investigator

Title/Position

Company Name or Institute

Department

Street Address

City

State or Province

Country

Zip or Postal Code

Email Address

(Area Code) Telephone Number

(Area Code) Facsimile Number

Contact Person (if different from Authorized Representative)

Email Address

(Area Code) Telephone Number

BILLING INFORMATION

The complete Kinex™ KAM-850 Antibody Microarray Kit is offered to allow clients to track the expression and phosphorylation of cell signalling proteins with ~850 pan- and phosphosite-specific antibodies in two (2) cell/tissue lysate samples with duplicate measurements on one chip with all of the supporting reagents. The Kinex™ KAM-850 Antibody Microarray Chip and Supporting Reagents are also available separately. Clients have the option of sending the processed KAM-850 microarray to Kinexus to obtain a high quality TIFF image of the scanned chip with dye labeled proteins. There is no additional fee for the scanning service. Kinexus can also provide a Kinex™ KAM-850 Antibody Microarray Analysis Report for quantification of the scanned chip for an additional US\$195 upon completion of a KAM-850 Analysis Report Service Order Form (KAMR-SOF), which is provided with the Kit.

INTRODUCTORY PRICE – US \$749 PER COMPLETE KAM-850 ANTIBODY MICROARRAY KIT

Total # of Complete Kinex™ KAM-850 Antibody Microarrays Kits ordered: _____ @ US \$749 per complete kit = \$
Total # of Kinex™ KAM-850 Antibody Microarrays Chips ordered: _____ @ US \$499 per chip = \$
Total # of Kinex™ KAM-850 Supporting Reagent Kits ordered: _____ @ US \$299 per reagent kit = \$

Quotation or Reference Number: _____ - \$

TOTAL COST FOR THIS ORDER (excluding courier shipping costs) = \$

FOR CANADIAN CUSTOMERS ONLY:

Add an additional 12% to the above total for GST (No. 893907329 RT0001): + \$ _____ = \$

TOTAL AMOUNT PAYABLE IN U.S FUNDS

PAYMENT METHOD

PURCHASE ORDER ACCEPTED FROM COMPANIES AND INSTITUTES WITH APPROVED CREDIT. P.O. NUMBER: _____
 VISA OR MASTERCARD

Print Cardholder Name

Visa Number

Expires (M/Y)

Cardholder Signature

BILLING INFORMATION SEND INVOICE TO CUSTOMER AT ABOVE ADDRESS OR SEND INVOICE TO ACCOUNTS PAYABLE CONTACT: Dr Mr Ms

Accounts Payable Contact Name

Company Name or Institute

Street Address

City

State or Province

Country

Zip or Postal Code

(Area Code) Telephone Number

AUTHORIZATION

CUSTOMER AGREES TO PAY FOR THE ORDERED ITEMS AND ASSOCIATED SHIPPING COSTS. IN THE EVENT THAT AN ORDER IS CANCELLED FOLLOWING SHIPPING AND THE UNOPENED PRODUCT IS RETURNED WITHIN 1 MONTH, A US \$150 PER KIT RE-STOCKING FEE WILL APPLY.

Print Name of Authorized Representative or Principal Investigator

Authorized Signature

Date y/m/d

How did you originally hear about the KABM Product? Direct Mail Email Web Site Advertisement Referral Conference or Trade Show Other

High Content Antibody Microarray Competitor Analysis

Performed in-house by Kinexus Bioinformatics Corporation

Date: 2012 November 1

Company	Kinexus	FullMoon	Sigma	Hypromatrix	Raybiotech	R&D Systems
Array name	KAM-850 Antibody Microarray	Phospho Explorer Antibody Microarray	Panorama® Antibody Array – XPRESS Profiler725	Signal Transduction AntibodyArrayTM	RTK Phosphorylation Array 1	Human Phospho-Receptor Tyrosine Kinase Array Kit
Catalogue ID	KABM-K	PEX100	XP725	HM3000	AAH-PRTK-1-8	ARY001B
Total number of distinct antibodies	854	1318	725	399	0	49
Non-redundant - Pan antibodies (1)	362	354	579	395	71	49
Non-redundant Phospho antibodies (2)	287	283	27	2	0	0
Redundant - Pan antibodies	155	380	114	2	0	0
Redundant Phospho antibodies	50	301	5	0	0	0
Non-redundant Pan antibodies - Kinases (3)	189	113	75	70	71	49
Non-redundant Pan antibodies - Phosphatases (4)	31	6	10	10	0	0
Overlap of Phosphosite- and Pan-specific antibodies (1)	Almost no overlap	Almost complete overlap	Almost no overlap	Only 2 phosphosite-specific antibodies	Only generic phosphotyrosine-specific antibodies	Only generic phosphotyrosine-specific antibodies
Number of chips (5)	1	2	2	1	1	1
Number of fields per chip (6)	2	1	1	1	8	4
Number of cell/tissue lysates testable (6)	2	2	2	1	8	4
Number of replicates per field	2	2	2	1	2	2
Total number of antibody spots and configuration per kit	1 x 854 x 2 x 2 = 3,416	2 x 1318 x 2 = 5,272	2 x 725 x 2 = 2,900	1 x 399 x 1 = 399	1 x 71 x 8 x 2 = 1,152	1 x 49 x 4 x 2 = 392
Spot diameter	150-200 µm	220-250 µm	300 µm	NA (7)	NA	NA
Median range in duplicates from average (8)	12%	4%	8%	NA	NA	NA
Dynamic range (9)	>5000-fold	1250-fold	1000-fold	NA	NA	NA
Human reactivity (10)	Yes	Yes	Yes	Yes	Yes	Yes
Array type	3D matrix coated glass slide	3D polymer coated glass slide	Nitrocellulose-coated Whatman FAST® glass slide	Nitrocellulose membrane	Option of membrane or glass chip	Nitrocellulose membrane
Recommended or provided detection	Non-competitive/ Single dye	Non-competitive/ Single dye	Competitive/ Two dye	Non-competitive/ Antibody	Non-competitive/Biotinylated Antibody	Non-competitive/HRP Antibody
Price - chip/array only (11)	\$499	\$1,300	NA	\$980	NA	NA
Price per antibody spot (11)	15¢	25¢	NA	\$2.45	NA	NA
Kit price - chip/array + reagents (12)	\$749	1,300 + 300 + 50 = \$1,650	1,930 + 100 = \$2,030	NA	\$1,030	\$495
Kit price per antibody spot (12)	22¢	31¢	70¢	NA	89.¢	\$1.26
Service price - chip/array report generation (13)	\$199	No	No	No	No	No
Service price per sample (13)	\$885*/\$1,498**	\$1,950	NA	NA	NA	NA
Service Cost per sample per spot (13)	52¢*/88¢**	74¢	NA	NA	NA	NA
Citations via Google Scholar (14)	65	17	11	13	13	27
Antibody microarray database access	Yes (15)	No	No	No	No	No
Follow-up Western blotting service	Yes (16)	No	No	No	No	No

Company	Kinexus	FullMoon	Sigma	Hypromatrix	Raybiotech	R&D Systems
Case Study 1: Samonella infected HeLa cells (17)	% of antibodies showing ≥50% changes from untreated control: $224/803=27.9\%$	% of antibodies showing ≥50% changes from untreated control: $6/1318=0.5\%$	NA	NA	NA	NA
Case Study 2: EGF treated A431 cells (18)	% of antibodies showing ≥50% changes from untreated control: $44/604=7.3\%$	NA	% of antibodies showing ≥50% changes from untreated control: $6/224=2.7\%$	NA	NA	NA

Notes

1. Non-redundant antibodies refers to those that target a particular protein or phosphosite. Redundant antibodies correspond to additional antibodies that target a protein for which a non-redundant antibody is already provided, but a different epitope in that protein may be selected. Pan-specific antibodies recognize the target proteins whether they are phosphorylated or not, except in the case of the Phospho Explorer Antibody Microarray from Full Moon. In this case, their pan-specific antibodies recognize only the dephosphorylated forms of the target proteins on their microarray. Consequently, these antibodies do not provide an accurate measure of the changes in total target protein levels due to expression changes.
2. Phospho-antibodies recognize distinct phosphorylation site sequences when they are phosphorylated. For some array, generic phospho-tyrosine, phospho-threonine and phospho-serine antibodies may also be included.
3. Number of distinct protein kinases for which pan-specific antibodies are available in the printed array.
4. Number of distinct protein protein phosphatases for which pan-specific antibodies are available in the printed array.
5. Number of glass slides or membranes provided for the price shown.
6. Number of distinct fields per slide. In a non-competitive, single dye-binding application, 1 sample would be analyzed per field. In a competitive, two dye-binding application, 2 samples would be analyzed per field. However, due to differential dye binding by proteins, it is necessary to perform another microarray analysis with identical samples and protocols, except the dyes are switched between the samples. The competitive, two dye-binding methodology is not recommended due to this problem and because of poorer dynamic range.
7. NA = Not available or not applicable
8. Median error range is estimated from the difference of duplicate antibody spot measurements with the average of recorded signals from these average spots. The percent error is generally reduced with stronger detected antibody spot signals.
9. Dynamic range is based on the highest and lowest detected signals that have percent errors within 20% of the recorded spot signal strength.
10. U.S. pricing is based on the purchase of only arrays without any support reagents if available from manufacturer. Shipping not included. Number of spots includes duplicates.
11. For the Full Moon microarray kits, supporting reagents must be purchased separately for \$300, but this does not include the cost of the dye for labeling proteins. We have added another \$50 to cover this. For the Sigma microarray kits, supporting reagents are included, but this excludes the purchase of two dyes, which would be about \$100 extra.
12. Clients can send processed microarray chips to Kinexus for generation of a Report that includes data analyses and identification of leads for follow-up.
13. Clients can send cell/tissue lysate samples to Kinexus and Full Moon for antibody microarray analyses as a service. Kinexus offers non-confidential* and fully confidential** pricing options. Non-confidential results may be placed in the open-access KiNET website after a 6 month hold.
14. Based on searches with Google Scholar that includes the name of the company and the specific kit. This approach may not capture all literature references, but it provides equitable search parameters. Over one hundred literature references to the Kinex™ antibody microarrays can be generated with broader search terms.
15. Kinexus provides open-access to its online KiNET Antibody Microarray database with the results from over 2000 different Kinex™ KAM analyses as well as Kinetworks™ multi-immunoblotting data from over 6000 other cell/tissue lysate samples from its KiNET Immunoblotting database.
16. Kinexus provides a custom multi-immunoblotting service in which 18 different leads from the Kinex™ KAM-850 Antibody Microarray can be confirmed by Western blotting for \$650. per sample with a 2-3 week turn-around.
17. About 19% of the phosphosite- and 18% of the pan-specific antibody targets on the Full Moon microarray are similarly tracked with the Kinex™ microarray. For this study, the Kinex™ KAM-800 Antibody Microarray chip was originally used. In a parallel study with similar cell lysates, Samonella was observed to induce significant changes in 24% of 9508 different phosphosites in 1973 HeLa cell proteins (Lindsay et al. (2011) Science Signaling 4 (191), rs9. [DOI:10.1126/scisignal.2001668]).
18. For this study, the Panorama® Cell Signaling (CSAA1) microarray and Kinex™ KAM-650 antibody microarrays were tested.

Summary

This competitor analysis clearly demonstrates that the Kinex™ KAM-850 Antibody Microarray is superior to all of its competitors with respect to:

- largest coverage of diverse non-redundant target proteins and phosphosites;
- largest total number of protein kinase and protein phosphatases targets;
- minimization of overlap of epitopes to permit highest pairing of phosphosite- and pan-specific antibodies;
- improved analyses with novel chemical cleavage of lysate proteins, non-competitive, single dye methodology, and usage of same chip for two samples ;
- greatest dynamic range of detection;
- lowest pricing per antibody spot regardless of whether clients purchase only the KAM-850 chip, the kit or utilize our convenient proteomics services;
- greatest validation in the scientific literature from the most published scientific reports using the Kinex™ antibody microarrays;
- online, open-access to the results of thousands of other studies using the Kinex™ antibody microarrays; and
- the ability to have lead results from the KAM-850 chips confirmed for clients with a convenient and economical Western blotting service.

It is unclear where the antibodies printed on the arrays of our competitors are sourced from, and in some cases, they state that they will not reveal this. However, our antibodies have been cherry-picked from over 25 different vendors, and selected from the top 25% of all of the more than 4500 antibodies independently and stringently tested by Kinexus. We are pleased to disclose the commercial sources of antibodies that prove to be interesting and useful to our clients. We are happy to assist our clients in planning their studies and helping to interpret their results with our friendly and knowledgeable service representatives. Over 1650 scientists in over 35 countries world-wide have benefited from our powerful suite of proteomics and bioinformatics services. You can see more for less from Kinexus than from any of our competitors, and we have the data to prove it.



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