

Appendix B

High Content Antibody Microarray Competitor Analysis

Performed in-house by Kinexus Bioinformatics Corporation

Date: 2015 February 15

Company	Kinexus	Full Moon	Hypromatrix
Array name	KAM-880 Antibody Microarray	Phospho Explorer Antibody Microarray	Signal Transduction AntibodyArray™
Catalogue ID	KABM-KAM880	PEX100	HM3000
Total number of distinct antibodies (1,2)	877	1318	399
Non-redundant - Pan antibodies (3)	374	354	395
Non-redundant Phospho antibodies (4)	283	283	2
Redundant - Pan antibodies	192	380	2
Redundant Phospho antibodies	28	301	0
Total number of antibody spots and configuration per kit (5,6)	1 x 877 x 2 x 2 = 3,508	2 x 1318 x 2 = 5,272	1 x 399 x 1 = 399
Median range in duplicates from average (7,8)	9%	4%	NA
Dynamic range (9)	>6000-fold	1250-fold	NA
Array type	3D matrix coated glass slide	3D polymer coated glass slide	Nitrocellulose membrane
Recommended or provided detection (6)	Non-competitive/ Single dye	Non-competitive/ Single dye	Non-competitive/ Antibody
Kit price - chip/array + reagents (10)	\$749	1,380 + 320 + 50 = \$1,750	980 + 200 = \$1,180
Kit price per antibody spot	21¢	33¢	295¢
Service price - chip/array report generation (11, 12)	\$249	No	No
Citations via Google Scholar (13)	147	85	40
Antibody microarray database access (14)	Yes	No	No
Follow-up Western blotting service (15)	Yes	No	No
Case Study: Samonella infected HeLa cells (16)	% 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

Notes

- The KAM-880 chip features 307 pan-specific and 200 phosphosite-specific antibodies for protein kinases.
- The KAM-880 chip features 65 pan-specific and 4 phosphosite-specific antibodies for protein phosphatases.
- 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.
- Phospho-antibodies recognize distinct phosphorylation site sequences when they are phosphorylated. For some arrays, generic phospho-tyrosine, phospho-threonine and/or phospho-serine antibodies may also be included.
- The first number refers to the number of glass slides or membranes provided for the price shown.
- 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. Number of spots includes duplicates.
- NA = Not available or not applicable
- Median error range is estimated from the difference of duplicate antibody spot measurements with the average of recorded signals from these spots. The percent error is generally reduced with stronger detected antibody spot signals.
- Dynamic range is based on the highest and lowest detected signals that have percent errors within 20% of the recorded spot signal strength
- The first number for the Full Moon and Hypromatrix kits refers to just the U.S. purchase of only arrays without the support reagents that come standard with the KAM-880 kit. For the Full Moon microarray kits, supporting reagents must be purchased separately for an additional \$320, but this does not include the cost of the dye for labeling proteins. We have added another \$50 to cover this. For the Hypromatrix membranes, no supporting reagents are included, so we have conservatively added another \$200 to cover the detection reagents. The extra shipping costs for additional supporting reagents from other supplier is not included.
- Clients can send processed microarray chips to Kinexus for generation of a Report that includes data analyses and identification of leads for follow-up. The KAM-880 Report also includes Kinexions Pathway Analysis with over 60 graphic maps that are directly linked to the client's results.
- 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.

Notes

13. 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 and fifty literature references to the Kinex™ antibody microarrays can be generated with broader search terms.
14. 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.
15. 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.
16. About 19% of the phosphosite- and 18% of the pan-specific antibody targets on the Full IMoon 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]).

Summary

This competitor analysis clearly demonstrates that the Kinex™ KAM-880 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 separate cell/tissue lysate 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-880 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 6000 antibodies independently and stringently tested by Kinexus. Moreover, about a quarter of the antibodies used in the KAM-880 chip were developed in-house at Kinexus and are rigorously tested and available in 25 µg amounts for less than US\$90. 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 1700 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 lots of data to prove it.



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