



KINETWORKS™ MULTI-IMMUNOBLOTTING

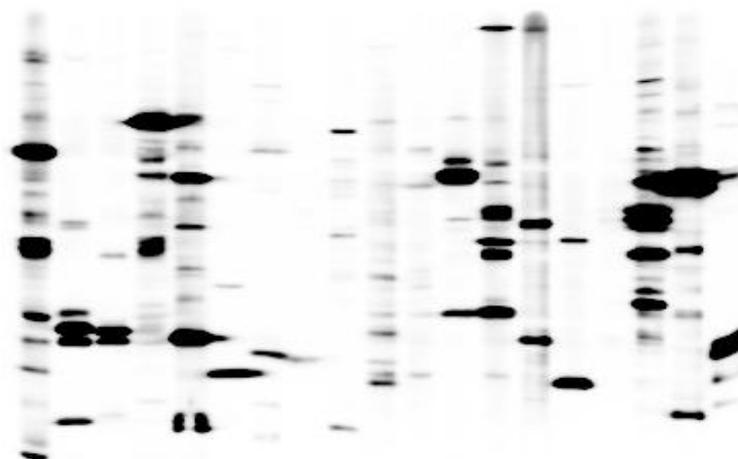
1. INTRODUCTION

The Kinetworks™ signal transduction protein profiling services are a convenient, reliable and cost-effective solution to assist scientists in the discovery of productive research leads. These services utilize a proprietary technology based on multi-immunoblotting that generates a unique identification pattern for each sample analyzed and can provide information about the quantitative expression level for each protein detected, its covalent modification, its subcellular location and the identification of protein-protein interactions. It is highly accurate, since the detection of a target protein is based on its immunoreactivity and apparent molecular mass. Kinexus has undertaken the testing of more than 3,000 commercial antibodies to select the most potent and specific antibodies for detecting low abundance proteins over a wide range of model systems. The Kinetworks™ approach, which has been under development and field-tested for over eight years, is faster and more sensitive for specific protein detection and offers greater versatility and reproducibility than many other proteomics methods. Presently, with over 800 antibodies Kinexus can track more than 650 cell signalling proteins and phospho-sites and several hundred unknown cross-reactive proteins. Only our Kinex™ antibody microarray services provide a cheaper alternative to profiling changes in protein expression and phosphorylation than our Kinetworks™ protein profiling, but the microarray approach is less accurate and generates a high degree of false positives and false negatives. Our Kinetworks™ Custom Antibody Screen (KCPS 1.0) is the recommended route to validate interesting results that are generated from our Kinex™ microarray services.

Kinexus currently offers 6 different standard analytical signal transduction protein profiling services. The lists of target proteins tracked in each standard screen are referenced in Appendix B-G. In addition to our standard screens, we offer 2 flexible custom screening services including the Kinetworks™ Custom (Multi-Antibody) Protein Screen (KCPS 1.0) that allows clients to choose *any* 18 antibodies of interest out of more than 650, which are listed in Appendix H. The Kinetworks™ Custom (Multi-Sample) Screen (KCSS 1.0) allows clients to choose up to 3 target proteins (of diverse molecular weight) quantitated in 8 different samples side by side on the same immunoblot. The Kinex™ antibody microarray service KAM-1.1 tracks about 650 signalling proteins in duplicate in two samples on the same microarray slide. For more information about this service, please review the Kinex™ Customer Information Package available on-line from our website at www.kinexus.ca. Clients can compare and correlate their experimental results with our Kinetworks™ services with thousands of other Kinetworks™ analyses that Kinexus has performed over the last 9 years by querying our KiNET databank on-line (www.kinexus.ca/kinet) .

IMPORTANT NOTICE – Our Kinetworks™ analyses of over 10,000 cell/tissue samples have revealed that there is a high degree of variability in the expression and phosphorylation levels of signal transduction proteins in diverse cell and tissue type that is species, organ, tissue and gender dependent. Consequently, we recommend that our standard Kinetworks™ Screens be performed initially to ascertain which target proteins are detectable in your experimental model systems before conducting any Custom Screens. All experimental results should be reproduced prior to publication in accordance with good laboratory practices.

Kinexus provides both qualitative and semi-quantitative analyses of the expression and phosphorylation states of protein kinases and cell signalling proteins in cell and tissue samples as part of the Kinetworks™ screening service. The qualitative analyses include TIFF files of the immunoblots that feature the detected target signalling proteins (see example of a Kinetworks™ immunoblot image below). The Kinetworks™ analysis has been specially optimized to reveal band shifts in signalling proteins on SDS-PAGE gels that may arise from their phosphorylation. The quantitative analysis of the strength of the enhanced chemiluminescence signal for each target protein is provided in a Microsoft Excel spreadsheet. For multiple samples within the same profiles, Kinexus provides Comparison Reports for the target proteins and graphs the data against the control samples. The Kinetworks™ screening service is unmatched for the information that it provides about multiple kinase expressions and phosphorylations in a single assay. To view example images or a sample Kinetworks™ Report, please visit our website at www.kinexus.ca and select the links “Our services” and “Kinetworks™”. All the Kinetworks™ Screens have been optimized to perform in human, mouse and rat model systems, but can also work for many protein targets in cow, pig, dog, rabbit, chicken, frog, starfish and other various model systems.



An example of a KPSS 1.3 multi-immunoblot pattern.