Inhibitor Screening Utilizing Human Kinase Multiplex Arrays

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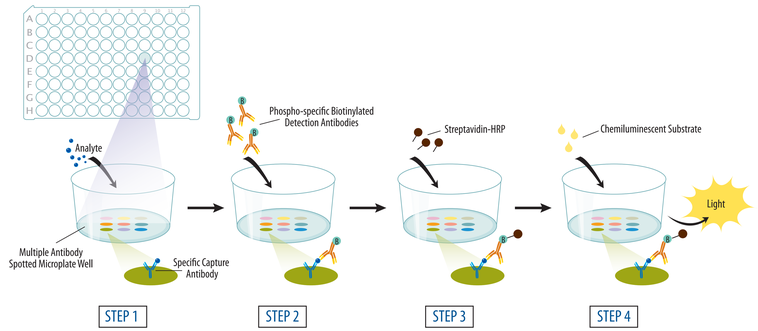
ABSTRACT

Protein kinases regulate many different signaling pathways and cellular processes. They have been implicated in various cancers, typically displaying increased activation, and are now being investigated as potential therapeutic targets. Thus, while investigating potential anti-cancer drugs, it would be beneficial to utilize assay techniques that allow a high-throughput analysis of kinase phosphorylation. The Proteome Profiler™ *96* Human Phospho-Kinase Array is a microplate-based multiplex immunoassay that employs a two-site sandwich immunoassay technique to simultaneously measure changes in the phosphorylation of multiple intracellular kinases. This assay utilizes a 96-well microplate pre-spotted with a series of antibodies to immobilize different kinases present in a sample. A cocktail of phospho-specific biotinylated detection antibodies and Streptavidin-HRP are subsequently used to detect the bound proteins. A chemiluminescent substrate mix and a camera imaging-system are used to determine the relative amount of analyte bound in individual spots.* This plate-based multiplex assay was used to monitor kinase phosphorylation in the MDA-MB-453 breast cancer cell line following treatment with a kinase inhibitor library. MDA-MB-453 breast cancer cells exhibited basal hyperphosphorylation of Akt and GSK-3β. Akt activation was selectively inhibited by SB-203580, SB-202190, ML-7, and BML-265. The data were verified by singleplex ELISA. This study demonstrates that results obtained using the Proteome Profiler *96* Human Phospho-Kinase Array are comparable to data generated using a single analyte analysis technique, such as ELISA. In addition, this multiplex immunoassay offers the advantages of being highly sensitive while requiring small sample volumes, simultaneously detecting multiple analytes, and being suitable for high-throughput analysis.

*Suitable imaging systems include Quansys Biosciences Q-View™ Imager; Alpha Innotech Fluorchem® HD2 and FC2; BioRad® VersaDoc™ 4000 or ChemiDoc™ XRS; Fujifilm LAS-3000 or LAS-3000 Mini; Aushon Biosystems SearchLight®. Free analytical software is available from Quansys Biosciences.

ASSAY PROCEDURE



SPOT LAYOUT IN EACH WELL

PS112_pp96poster_DEC

FIGURE 1

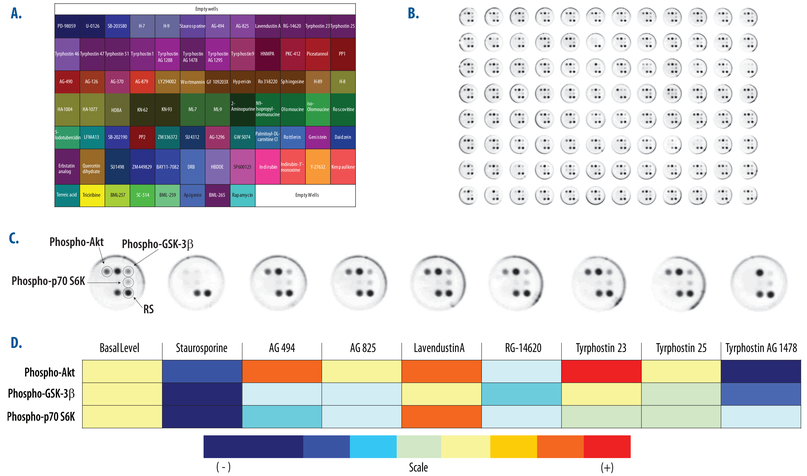


Figure 1. Profiling changes in kinase phosphorylation following treatment with various inhibitors. The MDA-MB-453 human breast cancer cell line was grown in a 96-well tissue culture plate to ~ 95% confluency. Cells were incubated for 4 hours with 20 µm of one of 80 different kinase inhibitors (**A**) from a kinase inhibitor library (Enzo, Catalog # BML-2832-0100). Cells were lysed, and dilutions of the lysate were transferred to a Proteome Profiler *96* Human Phospho-Kinase Array (Catalog # ARZ004) plate. **B.** An image of the wells was acquired on a Q-View Imager. **C.** An image of 9 wells captured with the Q-View Imager. **D.** Heat maps for Phospho-Akt, -GSK-3β, and -p70 S6K were generated using the pixel densities obtained from image analysis software.

FIGURE 2

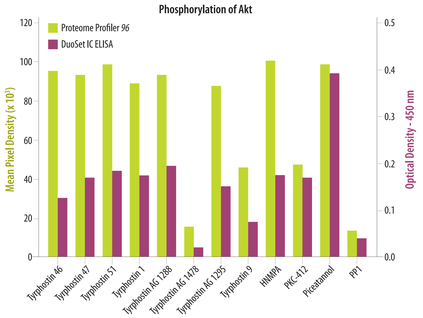


Figure 2. Comparison of Phospho-Akt signals detected using the Proteome Profiler *96* Human Phospho-Kinase Array and the DuoSet® IC ELISA. The MDA-MB-453 human breast cancer cell line was treated with different kinase inhibitors. Phosphorylation of Akt in cell lysates was assessed using the Proteome Profiler *96* Human Phospho-Kinase Array (Catalog # ARZ004) and the Human/ Mouse/Rat Phospho-Akt (Pan) (S473) DuoSet IC ELISA (Catalog # DYC887). Mean pixel densities obtained from the Proteome Profiler *96* Array (green bars) and optical densities obtained from the DuoSet IC ELISA (purple bars) are shown. Results obtained using the two detection methods are comparable.

FIGURE 3

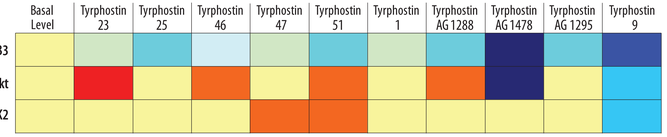


Figure 3. Profiling changes in phosphorylation of ErbB3, Akt, and ERK1/ERK2 following treatment with different kinase inhibitors. The MDA-MB-453 human breast cancer cell line was treated with different kinase inhibitors. Phosphorylation of ErbB3 in cell lysates was assessed using the Proteome Profiler *96* Human Phospho-RTK Array 2 (Catalog # ARZ002), while Akt and ERK1/ERK2 phosphorylation was assessed using the Proteome Profiler *96* Human Phospho-Kinase Array (Catalog # ARZ004). Using the results of a single experiment, heat maps for Phospho-ErbB3, -Akt, and –ERK1/ERK2 following treatment with 10 different Tyrphostin kinase inhibitors were generated using the pixel densities obtained from image analysis software.

FIGURE 4

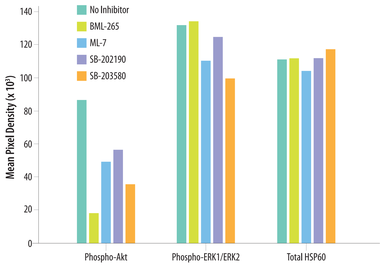


Figure 4. Comparison of Phospho-Akt and -ERK1/ERK2 signals following treatment with select Akt inhibitors. The MDA-MB-453 human breast cancer cell line was treated with select Akt inhibitors. Phosphorylation of Akt and ERK1/ERK2 was assessed using the Proteome Profiler *96* Human Phospho-Kinase Array (Catalog # ARZ004). Histogram profiles were generated by quantifying the mean spot pixel densities from individual antibody spots using analytical software. Total HSP60 protein levels were assessed to monitor sample loading.

FIGURE 5

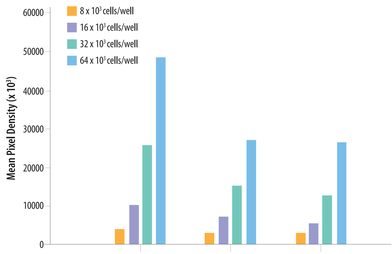


Figure 5. Comparison of HSP60 protein levels in different breast cancer cell lines. The MCF-7, MDA-MB-453, and T47D human breast cancer cell lines were grown in tissue culture flasks to ~95% confluency. Cells were trypsinized, counted, and then plated in a 96-well plate at the indicated densities. After 24 hours, cell lysates were assessed for total HSP60 using the Proteome Profiler *96* Human Phospho-Kinase Array (Catalog # ARZ004). Histogram profiles for HSP60 in the different cell lines were generated by quantifying the mean spot pixel densities from individual antibody spots using analytical software. The data demonstrate the ability to monitor sample load.

CONCLUSIONS

- Proteome Profiler *96* Human Phospho-Kinase Array allows multiple intracellular kinases to be monitored simultaneously in the same well.
- Proteome Profiler *96* Arrays offer the advantage of requiring minimal sample volumes.
- Results obtained using the Proteome Profiler *96* Arrays are comparable to the results obtained using a single analyte ELISA.
- This screening process can allow for the rapid selection of several kinase inhibitors for further analyses.

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