Analysis of Changes in RTK Phosphorylation Using Proteome Profiler™ *96* Antibody Arrays

David J. Finkel, Wade M. Johnson, Christopher J. Buehl, Rebecca A. Coleman, Maria T. Campos, Stefan M. Luhowskyj, Kristina M. Boldin, Steven P. Stoesz, Roberto Campos-Gonzalez, Richard A. Krzyzek, Kathryn M. Brumbaugh

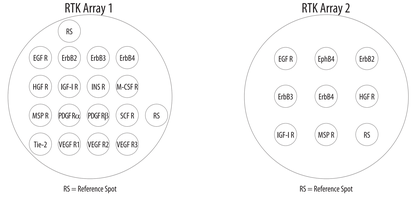
Poster 5572

ABSTRACT

Receptor-tyrosine kinases (RTKs) are transmembrane proteins that have been implicated in various cancers and are considered therapeutic targets. The phosphorylation of RTKs on tyrosine residues leads to their activation. Therefore, methods to identify the effect of RTK inhibitors or modifying antibodies on RTK phosphorylation are effective tools for drug screening. The Proteome Profiler *96* Human Phospho-RTK Antibody Array is a unique and powerful, plate-based multiplex immunoassay that utilizes ELISA techniques to measure changes in the phosphorylation of multiple RTKs simultaneously. In this assay, antibody/antigen reactions take place on the surface of a microplate that has been pre-spotted with antibodies against each RTK. Each spot corresponds to a unique analyte. A horseradish peroxidase-conjugated anti-Phospho-Tyrosine detection antibody is subsequently added to each well. A chemiluminescent substrate mix and a camera imaging system are used to determine the relative amount of analyte bound in individual spots. Experiments were performed by incubating the MDA-MB-453 breast cancer cell line with specific kinase inhibitors or with antibodies to ErbB receptors prior to NRG1-β1/HRG1-β1 treatment. Cells were lysed directly in 96-well plates and transferred to the Proteome Profiler *96* Human Phospho-RTK Array for analysis. NRG1-β1/HRG1-β1-dependent tyrosine phosphorylation of all four ErbB receptors was monitored simultaneously and the effects of different kinase inhibitors or modifying antibodies were determined. Proteome Profiler *96* Antibody Arrays offer the advantages of small sample and volume size requirements with as little as 5 µg of total cellular protein in a total volume of 100 µL being required, while still offering the specificity and sensitivity of sandwich immunoassays.

* Suitable imaging systems include Quansys Biosciences Q-View™ Imager; Alpha Innotech Fluorchem HD, SP, 8000, 8900, 9900, HD2, FC2; BioRad Versa Doc 4000 or XRS; Fujifilm LAS-3000 or LAS-3000 Mini. Free analytical software is available from Quansys Biosciences.

SPOT LAYOUT IN EACH WELL



ASSAY PROCEDURE

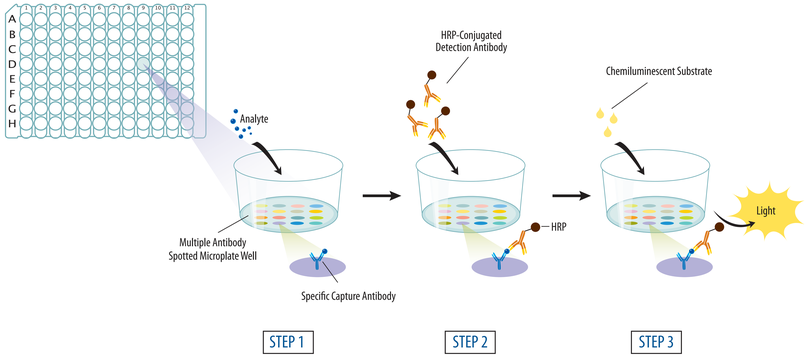


Figure 1.

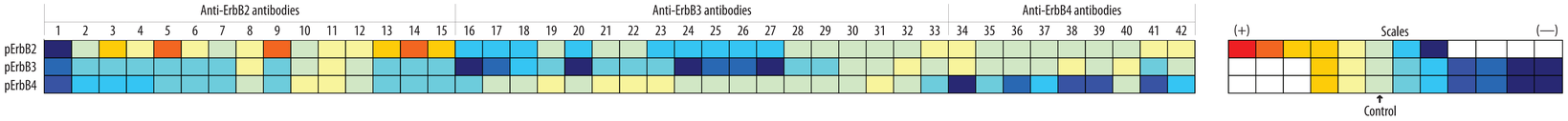


Figure 1. Profiling the changes in NRG1-β1/HRG1-β1-mediated RTK phosphorylation after treatment with Anti-ErbB antibodies. The human breast cancer cell line, MDA-MB-453, was grown in a 96-well tissue culture plate to ~85% confluency. Cells were incubated with one of 42 different anti-ErbB2, ErbB3, or ErbB4 antibodies for 20 hours, followed by treatment with 100 ng/mL recombinant human NRG1-β1/HRG1-β1 (Catalog # 396-HB) for five minutes to induce tyrosine ErbB phosphorylation. Cells were lysed and dilutions of the lysate were transferred to a Proteome Profiler *96* Phospho-RTK Array 1 (Catalog # ARZ001) plate. An image of the wells was acquired on a Q-View Imager. Using the results of a single experiment, heat maps for Phospho-ErbB2, -ErbB3, and -ErbB4 were generated using the pixel densities obtained from image software analysis. Each rectangle represents the mean effect of a single antibody in duplicate wells. Control = NRG1-β1/HRG1-β1-treated cells with no antibodies added.

Figure 2.

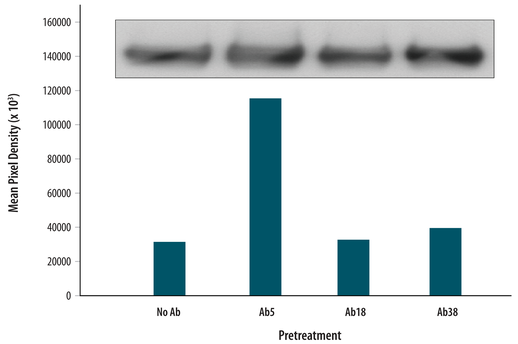


Figure 2. Comparison of Phospho-ErbB2 signals detected using the Proteome Profiler *96*-RTK Array 1 or using Western blotting. 5 µg of whole cell extracts from MDA-MB-453 cells incubated with or without anti-ErbB antibodies (pretreatment) and recombinant human NRG1-β1/HRG1-β1 as described above were run on ARZ001. Mean pixel densities of phospho-ErbB2 signals are represented by vertical bars. 30 µg of whole cell extracts were resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunoblotted with 1.0 µg/mL rabbit anti-ErbB2 (Y1248) AF1768 antibody (inset).

Figure 3.

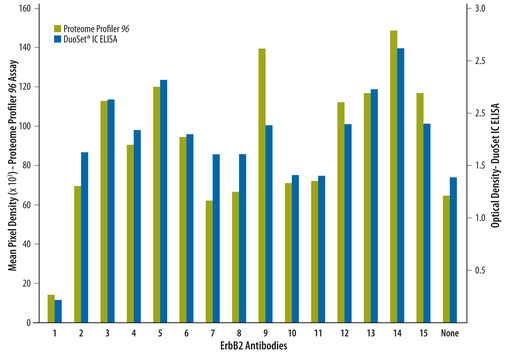


Figure 3. Comparison of Phospho-ErbB2 signals detected using the Proteome Profiler *96* Phospho-RTK Array 1 or using the DuoSet® IC ELISA. MDA-MB-453 cells were treated with different anti-ErbB2 antibodies. Phosphorylation of ErbB2 in cell lysates was assessed using the Proteome Profiler *96* Phospho-RTK Array 1 (Catalog # ARZ001) and the Human Phospho-ErbB2 DuoSet IC ELISA (Catalog # DYC1768). Mean pixel densities obtained from the Proteome Profiler *96* Antibody Array (green bars) compare well with optical densities obtained from the DuoSet IC ELISA (blue bars) are shown.

Figure 4.

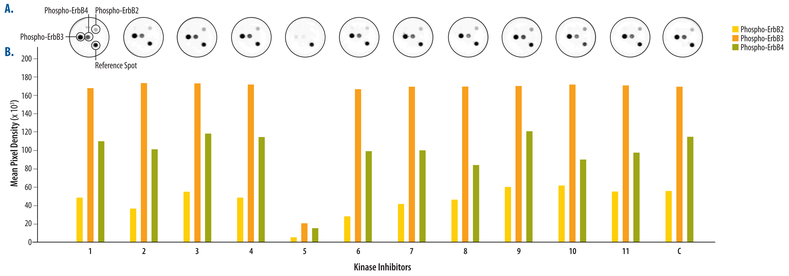


Figure 4. Profiling the effects of different kinase inhibitors on RTK phosphorylation. MDA-MB-453 cells were grown in a 96-well tissue culture plate to ~85% confluency before being treated with various kinase inhibitors at 33 µM for 1 hour prior to NRG1-β1/HRG1-β1 treatment. Cell lysates were analyzed using the Proteome Profiler *96* Phospho-RTK Array 2 (Catalog # ARZ002). Images of individual wells (**A**) and mean pixel densities of phospho-ErbB2, -ErbB3, and -ErbB4 (**B**) are shown.

CONCLUSIONS

- Proteome Profiler *96* Human Phospho-RTK Antibody Arrays allow up to 16 RTKs to be monitored simultaneously.
- Proteome Profiler *96* Antibody Arrays offer the advantages of small sample and volume size requirements, with as little as 5 µg of total cellular protein in a total volume of 100 µL being required.
- Results obtained using the Proteome Profiler *96* Antibody Arrays are comparable to the results obtained using a single analyte ELISA and more sensitive than conventional Western blotting.
- This screening process can allow for the selection of several anti-ErbB antibodies and inhibitors for further analyses.