Receptor Tyrosine Kinase Stimulation of Signaling Cytosolic Kinases

Pathway Analysis of EGF Stimulation of A431 and MCF-7 Cell Lines

On a single plate, samples of A431 and MCF-7 cells were analyzed for the activation states of 7 kinases as illustrated. Using 0.1 µg, 1 µg or 5 µg of cell lysate in each well and using different biotinylated antibodies specific for each kinase, A431 and MCF-7 cell lysates were analyzed for phospho- and total forms of 7 kinases, allowing the analysis of groups of analytes at the same time under the same conditions. Monitoring changes in the expression of analytes due to stimulation by mitogens or specific agents such as receptor ligands provides significant advantages to drug discovery and development.

Materials and Methods

Antibody Pairing Determination

To determine antibody pairing for each analyte, a single concentration was performed for each analyte to be paired to determine the best antibody pairing for each analyte. Following this, as many as 16 analytes could be performed on a single single assay plate. Lysates from multiple unstimulated tumor cell lines were compared with those from the same cell lines specifically stimulated with ligands to several well-characterized RTKs.

Quantitative Assessment of MEK-ERK Heterodimer (Total Protein)

The AMMP assay was used to demonstrate the detection of phosphorylated forms of several analytes in the MEK-ERK heterodimer, the most extensively studied pathway in cancer. To perform each kinase analysis, a single concentration was performed for each analyte to be paired to determine the best antibody pairing for each analyte. Following this, as many as 16 analytes could be performed on a single single assay plate. Lysates from multiple unstimulated tumor cell lines were compared with those from the same cell lines specifically stimulated with ligands to several well-characterized RTKs.

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