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Commentary

# Reverse Phase Protein Lysate Microarray and its Applications

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## DESCRIPTION

Reverse Phase Protein Lysate Microarray (RPMA) is a protein microarray developed as a dot-blot platform that enables quantitative evaluation of protein expression levels in several biological samples at once.

In order to determine the expression of the target protein across numerous samples, tiny amounts of cellular lysates, from intact cells and bodily fluids such as serum, urine, saliva, etc., are immobilized on individual spots on a microarray. One microarray can hold hundreds to thousands of samples that are printed in a series of replicates, depending on the design. Detection is carried out using chemiluminescent, fluorescent, or colorimetric techniques. Data quantification is performed by image capture of an array.

Multiple arrays spotted with the same lysate can be probed with various antibodies at the same time to accomplish multiplexing, which can then be used to construct a quantitative calibrated test. RPMA use whole-cell, undissected or microdissected cell lysates and can give precise data on post-translationally changed proteins that are not possible to obtain with other methods. As a result, RPMA offers high-dimensional proteomic data in an efficient, sensitive, and quantitative way. The antibodies employed in RPMA must therefore undergo western blot validation for specificity and efficacy against cell lysates.

RPMA has a number of applications, including the quantitative measurement of protein expression in tissues, body fluids, and cancer cells for biomarker profiling, cell signaling analysis, clinical prognosis, and diagnosis and treatment prediction. This is possible because an RPMA has been built to determine the differential expression of a protein marker level in a single experiment using lysates from various cells of one or more patients. It is also used to track over time change in proteins and its response to different pharmacological dosages. Exploring and mapping protein signaling networks and assessing molecular therapeutic targets and are some more uses for RPMA. Additionally, it has been proposed as a possible early screening test for cancer patients to aid with or direct therapeutic decision-making.

Other protein microarrays include Antibody Microarrays (AMAs) and forward Protein Microarrays (PMAs). Individually purified and occasionally denatured recombinant proteins are immobilized by PMAs on the microarray before being examined by antibodies and other small molecules. Antibodies that capture analytes from the sample placed on the microarray are immobilized by AMAs. Either direct labeling or a secondary labeled antibody against a distinct epitope on the analyte target protein is used to detect the target protein. PMAs and AMAs can both be categorized as forward phase arrays as both involve in immobilizing bait in order to capture an analyte. In forward phase arrays, numerous analytes in a test sample such as a cellular lysate or a patient's serum are evaluated simultaneously while the arrays are incubated with a single test sample.

As with all immunoassays, RPMAs major drawback is that it relies on antibodies to identify proteins. There are currently a small but quickly expanding number of signaling proteins for which analyzable antibodies are available. Additionally, before starting the RPMA analysis, it could be necessary to thoroughly screen a large number of antibodies by western blotting. RPMAs do not resolve protein fractions by molecular weight, in contrast to western blots. Therefore, it is crucial to do antibody validation.

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