







REVERSE PHASE PROTEIN ARRAY (RPPA) SERVICE

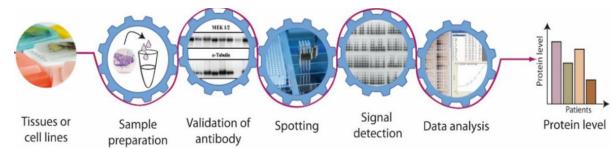
RELIABLE EVALUATION OF TARGET PROTEINS

RPPA

RPPA. high-throughput antibody-based microarray system with procedures similar to Western blots, is a super sensitive and precise technology for the quantitative measurement of hundreds of target proteins in both preclinical and clinical samples. This array format enables quantification simultaneous of proteins or phosphoproteins in multiple samples under the same experimental conditions. Furthermore, it is well-suited for signal transduction profiling of small numbers of cultured cells or cells isolated from human biopsies, including formalin-fixed and paraffinembedded (FFPE) tissues. Due to its simplified sample preparation (compared to mass spectrometry-based technologies) and its exceptional sensitivity for detecting lowabundance signaling proteins across a wide linear range, RPPA holds significant potential for characterizing deregulated interconnecting protein pathways and networks using limited sample amounts.

The knowledge of protein expression levels is crucial for predicting drug efficacy, estimating potential adverse effects, and identifying novel drug targets. Our highly optimized protein profiling services significantly accelerate the process of drug discovery and development for our clients while generating valuable data at low costs.

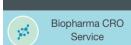
RPPA WORKFLOW



APPLIC

- Characterization of cellular signaling networks under various conditions
- Protein biomarker identification and validation in preclinical and clinical samples
- Drug target identification and validation
- Analysis of specificity of protein degradation by degrader compounds
- Mechanistic studies for various indications
- Determination of drug selectivity and identification of therapeutic targets
- Definition of regulatory mechanisms in signaling networks, including cross-talk and feedback loops













- Classification of patient tumors, prognostic determination, and response predictions to targeted therapies
- Understanding of pharmacodynamics and biologically relevant dosing and scheduling

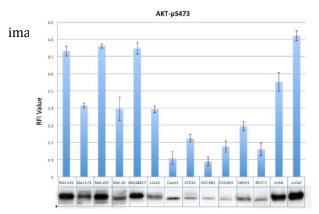
ADVANTAGES

- High multiplexity: Enables the detection and quantification of hundreds of distinct proteins, including their expression levels and modification status, using only 40 μ g of cell lysates (currently covering ~ 500 proteins from various signaling pathways).
- Cost-effective: This high-throughput approach allows for simultaneous testing and quantification of ~ 1000 samples on a single slide.
- High sensitivity: Exhibits greater sensitivity than ELISA while requiring minimal sample consumption.

RPPA VALIDATION WITH WESTERN BLOT

Below is a comparative analysis of AKT phosphorylation in cancer cell lines, utilizing both RPPA and Western blot techniques. Lysates from 14 cell lines cultured in complete media were subjected to analysis using RPPA and Western blot. The RPPA results are presented as relative fluorescence intensity (RFI) values displayed in a bar chart, alongside the corresponding raw western blot

- Quantitative: Applicable to recombinant proteins and phospho-peptides, allowing for precise quantification of protein expression and levels of modification.
- Simplified: No direct labeling or laborintensive depletion or fractionation of sample lysates is required.
- Versatile sample types: Various types of samples can be investigated, including those from cell cultures, live animals and patients (such as fine needle aspirates, laser capture microdissection, FFPE samples), sorted cells, as well as serum, plasma, saliva, urine, etc.



CURRENTLY AVAILABLE TARGETS

Our current RPPA testing can quantitate $\sim\!600$ well-validated protein targets, among which are $\sim\!100$ phosphoproteins of multiple signaling pathways. Please email support@axelabio.com for the target list.

AXELA BIOSCIENCES' SERVICE DELIVERABLES

- Spreadsheets containing sample identifiers and quantitated expression levels of each target proteins.
- Each dataset includes raw, normalized, and median-centered data, along with a pair of heatmaps (one supervised and one un-supervised).
- A study report to describe and summarize the results with data interpretation.