LC Sciences provides a comprehensive kinase analysis service utilizing high density protein kinase substrate (PKS) peptide microarrays based on PepArray™ technology for proteomic scale kinase profiling, quantitative measurement of kinase kinetic activities, and drug discovery research.

Perform protein kinase reactions on arrays of comprehensive sets of substrates. Measure kinase activity and inhibitor effects. Obtain hundreds of reaction profiles and curves in parallel for parameters such as $V_{\text{MAX}}$, $K_M$, and $K_I$. Use of microarray titer plate format will increase your workflow by at least 40-fold that of a single microtiter plate.

- **Protein Kinase Substrate Profiling** – Large scale mapping of kinase substrate specificity, detecting and discovering novel kinases, and validating computationally predicted or other types of kinase substrate peptide candidates.

- **Quantitative Protein Kinase Kinetics Studies** – performing quantitative measurement of protein kinase kinetic activities (PKA studies).

- **Drug Development** – Screening inhibition of kinase enzymatic activities by competition assays, identifying substrate peptides as biomarkers, and studying signaling pathways using multiplex reactions
LC Sciences provides a comprehensive kinase substrate profiling service to make both qualitative and quantitative measurements of kinase substrate phosphorylation. Screening on a peptide microarray (PepArray™) offers the opportunity to study thousands (up to 30K peptides/chip) of potential epitopes in a single experiment utilizing only sub-µg quantity of protein. Using a tiling method, we can systematically map the binding sites on a pathogen/protein at single amino acid resolution.

**COMPREHENSIVE SAMPLE TO DATA SERVICE**

This is a comprehensive kinase substrate profiling service—send us your sample—we’ll synthesize a custom designed array, carry out the sample enzymatic assays you request, perform data collection and analysis, and deliver a results report to you. Our cost effective 1-stop solution can save you tremendous time and money and the use of a microarray titer plate format will increase your workflow to 40X that of a single microtiter plate.

**MICROFLUIDIC ARRAY PLATFORM**

These are not spotted arrays! A proprietary µParaflo® microfluidic biochip is used and custom peptide sequences are synthesized on-chip. The microfluidic technology produces a uniform distribution of the sample solutions on the array, ensures efficient sample-peptide contact and enhances binding reactions and stringency wash processes. The microarray chip consists of thousands of three-dimensional chambers and is a closed system. Under these conditions multiplex protein assays are carried out in a way much like in thousands of pico-liter tubes, enclosure keeps the proteins in a stable environment, in solution and protected from air–oxidation/contamination. The miniaturized system provides automation, sample/reagent-savings and simplicity in operation.

**HIGH THROUGHPUT FORMAT**

- Thousands of different peptide sequences are synthesized on one array (1 cm²)
- 1 4K peptide microarray = 40 X 96-well microtiter plates. We have chip designs up to 30K.
- Target thousands of peptide sequences at once - perform thousands of multiplex parallel assays at addressable chip locations.
- Generate hundreds of binding curves in a single experiment.

**QUANTITATIVE RESULTS**

- Generate information about specific probe sequences - sequences can be defined to each single amino acid residue and the interpretation of the assay results are taken directly from digital image read out of an addressable microarray.
- Achieve reliable and quantifiable results through the highly stringent design and use of negative and positive control references. False positive readings are minimized.

**FLEXIBLE DESIGN**

- Completely customizable design of peptides according to your needs. All arrays are synthesized to order.
- Choose your own customer specified sequences and layout, or sequences custom designed by LC Sciences.
- Quickly revise microarray design and content to keep experiments moving forward based on previous results.

**ULTRA-LOW SAMPLE CONSUMPTION**

- Miniaturized array features reduces consumption of valuable samples to the sub-nanoliter to picoliter level per reaction.
- Perform thousands of peptide assays per array using only sub-µg of protein.

www.lcsciences.com 1-888-528-8818
Peptide Concentration Variation On-Chip

Our unique synthesis chemistry makes it possible to vary the substrate density of reaction sites to create a gradient across the array. This array can generate the same amount of data equivalent to that of 40 conventional microtiter plates! This form of miniaturized multiplex parallel protein/antibody assays saves assay samples, reagents, labor and time in generating binding affinity or enzymatic reaction curves with a single experiment, allowing cross comparison of the systems assayed. The use of comparisons provides a more comprehensive picture and more reliable results.

Close up view of a µParaFlo® biochip

Pico-liter Scale Microarray Titer Plate

Peptide sequences are synthesized on the array at different molecular densities and thus are presented at different “concentrations”.

A 4000 feature microarray titer plate contains the molecular content equivalent to 40 microtiter plates.

Titration curves are obtained by concentration variations of the peptide substrates or the binding proteins. Similarly, time-curves and inhibition curves can be measured simultaneously for a large number of peptides.
LC Sciences Standard Kinase Array Content
- Covers the entire human kinome with substrates representative of all PK families.
- Contains over a thousand unique peptide substrates.
- Also contains corresponding negative controls (Ser/Thr/Tyr substituted by Ala) and replicates of each sequence.

Custom Content Kinase Arrays
- Microfluidic platform and in situ synthesis permit total customization of sequence content.
- Thousands of customer defined unique peptide substrates are synthesized per array. No minimum limit on the number of unique arrays synthesized.
- LC Sciences can assist with sequence design.

Example of Standard Content for a Protein Kinase Substrate Microarray

Characterization of a Novel Protein Kinase
The preferred binding pattern of an unknown kinase revealed it is a serine/threonine kinase and belongs to PKA family.
**Multiplex Protein Kinase Assays**

**Kinetic Studies of PKA Reaction**
Parallel measurements of reaction curve as a function of time and substrate concentration. These measurements lead to derivation of \( V_{\text{MAX}} \) and \( K_{M} \). Pico-liter titer plate arrays enable simultaneous measurement of hundreds of such curves increasing workflow efficiency 40x.

**Real-time Measurements**

- **LRRASLG**
- **Substrate**
- **Phosphorylation Signal (A.U.)**
- **Time (min)**
- **0** to **70**

**Microarray Titer Plate Measurements**

- **Substrate**
- **Phosphorylation Signal (A.U.)**
- **[Substrate] (µM)**
- **0** to **60**

**Measurement of Kinase Inhibition Curves**
A powerful tool for protein kinase inhibitor screen assays - A Single array assay measures multiple inhibitory constants on various PK substrate peptides, allowing comparison of specificity of PK inhibition and identification of potential major and off-site phosphorylation sites.

(D1, D2, D3 - peptide surface density)

**Table 1: IC50 Values**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>IC50 (µM)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>EYDELMR-D1</td>
<td>0.97</td>
<td>0.96</td>
</tr>
<tr>
<td>EYDELMR-D2</td>
<td>1.36</td>
<td>0.97</td>
</tr>
<tr>
<td>EYDELMR-D3</td>
<td>1.83</td>
<td>0.98</td>
</tr>
</tbody>
</table>

**Table 2: IC50 Values**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>IC50 (µM)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>EYDELMR-D1</td>
<td>0.71</td>
<td>0.99</td>
</tr>
<tr>
<td>EYDELMR-D2</td>
<td>1.86</td>
<td>0.96</td>
</tr>
<tr>
<td>EYDELMR-D3</td>
<td>4.17</td>
<td>0.93</td>
</tr>
</tbody>
</table>
Comprehensive Sample to Data Service

Sample QC
Appropriate sample requirements are established for each particular array application. Integrity of the received customer sample is determined via a thorough analysis process. Samples that do not meet requirements are flagged and notification is sent with a recommendation not to proceed with the microarray assays.

Chip Synthesis and Control Experiments
A standard or custom peptide microarray is synthesized on a high density peptide µParaFlo® microfluidic biochip. Each chip contains multiple synthesis control sites optimized for rigorous quality analysis.

On-Chip Binding or Enzymatic Assays
Multiplex assays are performed on a µParaFlo® microfluidics chip which ensures uniform flow of the protein samples through closed pico-liter chambers. The assay is controlled by optimized binding protocols and performed under temperature controlled conditions. Binding/Enzymatic experiments are monitored to achieve high quality and stringency.

Titer-Plate Binding or Enzymatic Assays
Peptides on reaction sites are synthesized according to pre-determined surface densities as "concentration" variation sites. Parallel protein assays on these titer-plate reaction sites provide hundreds of curves for binding or enzymatic/inhibitory activity measurements with minimal systemic variations.

Time and Concentration Dependent Measurements
Multiple measurements are performed to obtain variable plots using time or concentrations of protein, enzyme, inhibitor, or co-factor.

Microarray Scan and Data Extraction
Microarray images are carefully scanned for a balanced view. Numerical intensities are extracted for control, background, reference and test peptides.

Data Analysis
Basic data analysis includes background subtraction, control and reference signal guided data processing, list of detected signals and data averaging results. Optional in-depth analysis and comprehensive data processing services such as: consensus sequence analysis, statistical and pattern analysis, plot generation and kinetic/thermodynamic parameter calculation is available as requested based on your specific application. A summary of the analysis report is emailed. The complete data set is saved to a CD, which is shipped via overnight carrier.
Microfluidic Array Platform
The microfluidic technology produces a uniform distribution of the sample solutions on the array, ensures efficient sample-peptide contact and enhances binding reactions and stringency wash processes. The microarray chip consists of thousands of three-dimensional chambers and is a closed system. Under these conditions multiplex protein assays are carried out in a way much like in thousands of pico-liter tubes, enclosure keeps the proteins in a stable environment, in solution and protected from air–oxidation/contamination. The miniaturized system provides automation, sample/reagent-savings and simplicity in operation.

Multiple–Time Repetitive Assays on the Same Chip
Peptide microarrays can be used for time-, protein/antibody/enzyme concentration-, or co-factor/inhibitor-dependent measurements. The array can be used multiple times, each time assay is carried out and assay signals are collected by fluorescence scan. These assays generate a very large set of data for proteomic assays and valuable information which would take weeks and months to produce using a conventional single protein/antibody/ enzyme assay kit.

Kinetic Curve of PKA Reaction
- LRRXSLG series of peptides. Parallel measurements of hundreds of reaction curves.
- Kinetic curves on time courses at different concentration of substrate.
- PKA kinase reaction was performed at 30°C, 0-480 min w/0.2 mM ATP, 0.5 µg/mL PKA.
- Longer reaction time reaches higher product. At 120 min, reaction at all concentrations reaches the highest product yield (signal plateau).
Kinase Profiling Customer Publications


μParaflo® Peptide Microarray Synthesis Technology Articles
