Introduction:

The MultiPep 2 instrument is a pipetting robot that can synthesize peptides of up to 25 amino acids. The work area of the MultiPep 2 has exchangeable modules specific to either resin or membrane-based synthesis, amino acid derivatives, reagents and solvent positions for peptide synthesis. It's fully automated in-solution solid phase synthesis operation with an integrated heater and shaker improves the coupling efficiency of longer, more difficult sequences.

Applications of MultiPep 2:

CelluSpot Slide Spot Synthesis:

The synthesis of peptides on four dissolvable cellulose membranes (CelluSpots) at a synthesis scale range from 1-10 µmol per disk.

Create hundreds of identical glass slide arrays with up to 768 unique peptides per slide with spot volumes down to 100 nL.

The high throughput slide spotter robot can create up to 29 slides at the same time.

Standard slide format has a spot diameter of approximately 0.8 mm and a spot-to-spot centre distance of 1.2 mm.

SPOT Membrane Synthesis:

The parallel synthesis of up to 2400 peptides on four cellulose membranes (600 peptides/membrane) for the generation of peptide arrays.

Filter Plates – Resin Based Synthesis:

The synthesis of up to 384 peptides in four dedicated 96-well filter plates at a synthesis scale ranging from 1-10 µmol.

Multiple Columns – Resin Based Synthesis:

24 to 48 mini-columns (250 and 500 µL) from 1-15 µmol scale.

72 columns (2, 5, 10 and 20 mL) from 10-300 µmol scale.

48 columns (2, 5 and 10 mL) from 10-500 µmol scale.

Structural & Biophysical Core Facility

Greg Wasney

Manager, Structural & Biophysical Core Facility

Peter Gilgan Centre for Research & Learning
The Hospital for Sick Children
686 Bay Street, Rm. 21.9708
Toronto, ON. M5G 0A4

Email: *greg.wasney@sickkids.ca*

Office: 416.813.7209
Office Internal Ext. 307209
Lab: 416-813-7654 ext. 309442

http://lab.research.sickkids.ca/sbc-facility/

SickKids®
THE HOSPITAL FOR SICK CHILDREN

Prepared by Shina Hussain

The Hospital for Sick Children's

Structural & Biophysical

MultiPep 2 – Automated Parallel Peptide Synthesizer

CelluSpot Slide Spot Peptide Synthesis - CEM Corporation

- Peptide screening libraries for peptide-protein interaction, epitope mapping and receptor binding.
- Peptide array to screen human sera, cell lysates, characterize antibodies and enzyme substrates such as kinases.
- High throughput automation allows for the synthesis of up to 768 unique peptides in 2 x 384, 4 x 192 or 8 x 96 format on one inert white background foil coated glass microscope slide.
- SPOT Membrane Peptide Synthesis and Solid Phase / In-Solution Synthesis, including 96-well plates and various size column synthesis formats.

CelluSpot Slide Spot Screening Assay

Theoretical Background

Fmoc-Chemistry

Amino acids have two reactive groups, an amino group and a carboxyl group, which bind (couple) to form a peptide bond. The amino acid building blocks carry a transient protection group called Fmoc. Fmoc chemistry is used to prevent polymerization and ensures only one molecule binds to the peptide chain at a time. Other reactive groups on the side chains are masked by protection groups during synthesis.

Slide Spot Peptide Array Synthesis

The peptide is synthesized on a dissolvable cellulose support (CelluSpot membrane) containing an Fmoc-β-Alanin (non-cleavable) linker. Peptide synthesis is a step-by-step elongation of a peptide chain by coupling amino acid building blocks on a cellulose support. At each step of the synthesis, every chain is reacted with one molecule of the next activated Fmoc amino acid. The peptide is assembled from its carboxyl terminal to the amino terminal end (C to N-terminal direction). After every coupling reaction, the amino-protection group (Fmoc) is removed by a moderately weak acid (Fmoc deprotection reagent), whereas the side chain protection group remains Intact.

The process is repeated until the desired peptide is synthesized. After the peptide is synthesized, the side chain protection groups are removed from the peptide by treatment with a TFA Cleavage cocktail.

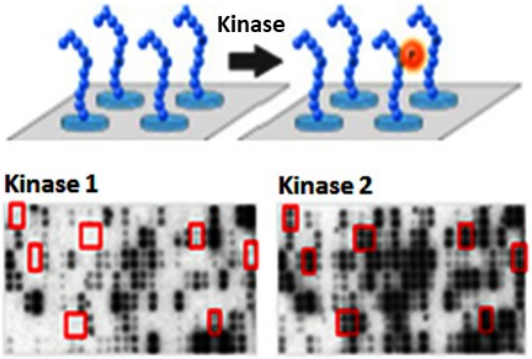
For a slide spot peptide array, the cellulose support (CelluSpot membrane) of the peptide is dissolved into a separate TFA solution. The solutions of individual peptides covalently linked to the macromolecular cellulose (peptide-conjugates) are spotted onto inert white background foil coated glass microscope slides. After evaporation of the solvent, a three-dimensional layer is formed which is not dissolvable in aqueous reagents used for standard assays.

Kinase-Substrate Assay

Kinase arrays with tyrosine- and serine/threonine-kinase substrates from annotated phosphorylation sites were synthesized on a dissolvable cellulose membrane (CelluSpot) and spotted onto white coated glass microscope slides using the Slide Spotter robot (CEM Corporation).

Determination of the substrate specificity of unknown or new kinases is done by changing the position of one or two amino acids in a peptide sequence.

Each kinase substrate was spotted in four replicates in each of the four identical subarrays contained on each slide. The kinase substrate array slides were immersed in a blocking solution to prevent non-specific binding. The kinase substrate arrays were incubated in two different kinase solutions to screen the substrate specificities of kinases or to identify auto-phosphorylation sites. Autoradiography was used for visualization.



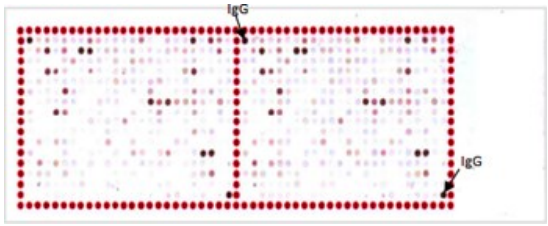
Comparison of Kinases

Some major differences in the comparison of substrate specificity for two different kinases are marked in red.

Epitope Mapping of Antigen

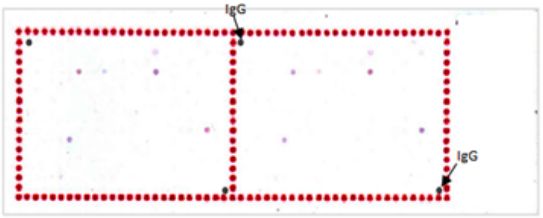
Several antigenic proteins of a pathogen were synthesized as overlapping sequence peptides on a dissolvable cellulose membrane (CelluSpot). The antigenic proteins were spotted in duplicate onto the white coated glass microscope slides. Each slide array also contains two human IgG feature peptides which were used as positive controls.

The peptide array slides were immersed in a blocking solution to prevent non-specific binding and incubated with infected human sera. The IgG antibody (specific to the disease of interest) binds to the interacting peptides and the presence of the bound IgG antibody was detected by using an HRP-conjugated anti-human IgG (secondary antibody). For enzymatic colour development, the slides were incubated in DAB substrate mixture until a brown enzyme colour developed.



Positive control (infected) Sera Sample

The IgG antibody interaction with some antigenic peptides were observed on both sides of the glass slide.



Negative control (healthy) Sera Sample

The same experiment was performed with the healthy human sera as a negative control.