Protein tyrosine phosphatase (PTP) microarrays

Jeroen van Ameijde, Rob Ruijtenbeek and Rob M. J. Liskamp

Utrecht University, PamGene International BV

Study Design
A new protein tyrosine phosphatase (PTP) microarray was developed based around a proprietary nitrophosphotyrosine residue. With this array product formation in a PTP assay was measured in real-time using routine microarray assay protocols. Many combinations of PTPs, substrates, inhibitors and biological samples were successfully studied with this technological setup.

Key Findings
The new nitrotyrosine residue can be incorporated into potential PTP substrates. A broad panel of PTPs recognizes the substrates. PTP activity can be uniquely measured in real-time through product formation. None of the drawbacks of measuring starting material consumption were observed, e.g. signal saturation issues, decreased sensitivity, competition between enzyme and detecting antibody and insufficient antibody binding at the start of the assay.
The assay protocol is comparable to and compatible with those used for other PamChip arrays.

“Author Quote”
The assay presented here uniquely combines a parallel, high-throughput nature, dynamic real-time measurement of product formation without the potential problems [...] measuring starting material consumption, and compatibility with complex biological matrices.

Background
Phosphatases and kinases act in tandem to regulate the crucial phosphorylation post-translational modification. Since many diseases are characterized by aberrant phosphate signalling, both enzyme families are high-potential drug targets. A lack of high-throughput assays, however, has held back investigation of phosphatases compared to kinases.
The unique new PTP array uses a proprietary nitro-phosphotyrosine building block which is recognized by PTPs and transformed into a nitrotyrosine residue which is selectively detected by an antibody. This allows a dynamic, real-time assay and circumvents known issues with detecting starting material consumption.

Conclusion
The new PamGene PTP platform uniquely offers real-time, dynamic measurement of PTP activity in a parallelized fashion and offers distinct advantages over assays which detect starting material consumption.

References:
PCT Int. Appl. WO2012/159997 A1