Positive pressure proteomics

Proteomics studies using mass spectrometry have an important role to play in understanding tumor cell biology and the impact of novel therapeutics. Sample clean-up prior to analysis is an essential part of these workflows, and researchers at Pfizer are performing this on a positive pressure workstation to save time and enhance reproducibility.

Pfizer is a leading pharmaceutical company with sites across the globe. One of its main areas of interest is oncology, and scientists in the Tumor Cell Biology group at the company's La Jolla facility in California are engaged in proteomics studies to gain a better understanding of the mechanism of action of drugs. A crucial part of these studies is mass spectrometry analysis of peptides, which depends on thorough sample clean-up to remove unwanted components that could interfere with the results. John Lapek, a principal scientist specializing in chemical proteomics and mass spectrometry, explained: "I joined the Tumor Cell Biology group about 18 months ago, having spent 10 years working in proteomics, including postdoctoral positions at the University

The Tumor Cell Biology group taking a well-earned break from the laboratory

of California, San Diego, and the Massachusetts General Hospital Cancer Center at Harvard Medical School. My focus is on chemical biology, which involves target deconvolution, engagement and occupancy studies, along with some broader mechanism of action investigations looking at global proteomic profiling to get a better understanding of how drugs work."

John continued: "Our proteomics workflows involve a complex digestion process, where cell lysates are typically treated with trypsin to break the proteins down into smaller, easier to analyze peptides, and a tandem mass tag (TMT) strategy that allows multiplexing of 11 samples. Before we can analyze the samples by mass spectrometry, we have to perform a couple of clean-up steps to remove urea and any other salts remaining after digestion, and excess, unreacted TMT reagents. This is done by solid phase extraction (SPE)."

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Manual SPE procedures tend to be time consuming and subject to operator-to-operator variation, which can be eliminated by automation. "In the past, sample clean-up was performed manually on a 16-place vacuum manifold. Not only is it very time consuming to pipette everything manually, but there will always be differences between the various users, as people don't load the columns at exactly the same rate or dry them to the same extent, and this affects the final analytical results. Automation was the way forward, as it would overcome these issues, allowing us to save time, increase throughput and improve reproducibility."

"I had been looking for an automated system for some time when I came across the Resolvex® A200 positive pressure SPE workstation at an American Society for Mass Spectrometry exhibition. Until then, I had only seen low throughput platforms that processed about eight samples at a time, and so my interest was immediately captured by this system's flexibility and potential to enable automated sample preparation in 96-well plates. We arranged a demo, and shortly afterwards a Resolvex A200 was set up in our lab for a one week trial. This allowed us to perform manual and automated sample preparation in parallel and compare the results."

"While the results of the manual and automated sample preparation were comparable, we found that the big advantage of using the Resolvex A200 was the amount of time we saved. Desalting used to be our biggest bottleneck; manually desalting 96 samples took two people dedicated to the task two days to complete. In contrast, the Resolvex A200 can process a 96-well plate in about an hour and a half. As we typically run three or four 96-well plates a week, it's a huge time saver. And if we have fewer samples to process, we also have the option to use individual columns. Another noticeable difference is the improvement in reproducibility, as automating our procedures has eliminated inter-operator variation. We have standard protocols set up on the system, which makes everything straightforward to operate and ensures that everyone uses the same method. It also means that if there is a query with a result, it is unlikely to have arisen due to variations in sample preparation."

"We've had the Resolvex A200 for over a year now, and find it easy to program and operate. It only took a couple of hours to show people how to use it, and we now have eight members of the group trained to run and program the system. Building on the success of automating our desalting protocols, we are considering using the system for simple fractionations – such as immunoprecipitation – in the future. This would remove the need to use spin columns. In addition, another one of our research groups has used the Resolvex A200 to investigate various metabolites. It lends itself to so many different applications," concluded John.

To find out more about Tecan's mass spectrometry sample preparation solutions, visit www.tecan.com/resolvex

To learn more about Pfizer's Tumor Cell Biology Group, go to www.pfizer.com/partners/candidate/oncology