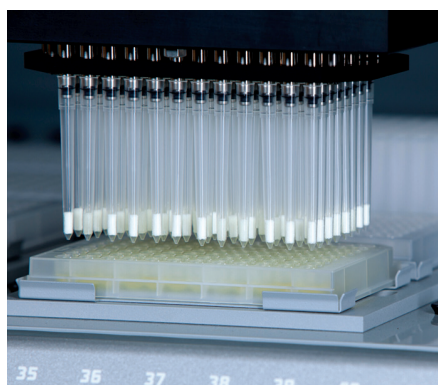


Large molecule bioanalysis simplified

Thermo Fisher Scientific is helping to drive biotherapeutic research with the development of innovative sample processing tools designed to simplify and accelerate analytical workflows. Offering automation-friendly affinity purification for proteins and antibodies, this provides a robust solution for high throughput preclinical research and development.

Biotherapeutics is now a key area of growth for the pharmaceutical sector, offering specificities and novel activities that cannot be achieved with small molecule drugs. The complex mode of action of many biotherapeutics requires extensive preclinical testing and characterization in cellular and animal models prior to clinical trials. Direct analysis of antibody-based biotherapeutics in complex biological samples is virtually impossible, due to the relatively low abundance of these proteins and significant interference from other macromolecules, requiring isolation of the antibody of interest prior to assessment.

Separation of these therapeutic molecules from complex media is generally performed using specific 'capture' antibodies affixed to a solid



Each microcolumn is mounted within a pipette tip for simplified liquid handling

surface, allowing the target protein to be immobilized and purified with repeated wash cycles. This is commonly performed by fixing the capture antibodies to a bead-based support suitable for either magnetic or vacuum-based separation, but these techniques can be time consuming – commonly taking seven to eight hours – and offer poor recovery rates, making quantitative analysis difficult. This approach is also difficult to standardize or automate in a high throughput format, leading to higher costs for method development and implementation.

“ This significantly increases the rate of immobilization... allowing the incubation time to be reduced by up to 75 % in some cases. ”

To overcome these limitations, Thermo Fisher Scientific has developed a solution which combines microfluidic principles with automated liquid handling to accelerate sample preparation and improve recovery rates for biological macromolecules.

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Eric Niederkofler, Research & Development Manager at Thermo Fisher Scientific, explained: “In the 1990s, we began the development of our MSIA™ (Mass Spectrometric Immunoassay) platform, which combined antibody capture of a protein of interest with mass spectrometric (MS) analysis. As antibody technologies and MS instrumentation have developed, the limitations of existing solid phase isolation techniques have become increasingly apparent, particularly with regards to sensitivity. As a result, we developed affinity microcolumns – small volume, high surface area supports mounted within pipette tips – as a way of improving both the ease of use and scalability of our MSIA technology. Each column is covalently derivatized with streptavidin, allowing straightforward immobilization of the user's choice of biotinylated capture antibodies directly onto the inner surfaces of the microcolumn.”

“Initially developed for use in our in-house research activities, this system increases the rate and efficiency of target binding by using repeated aspiration and dispense cycles. A key benefit of this 'active' incubation approach is that the microfluidic channels in the column repeatedly bring the target molecule into close proximity with the immobilized capture antibody. This significantly

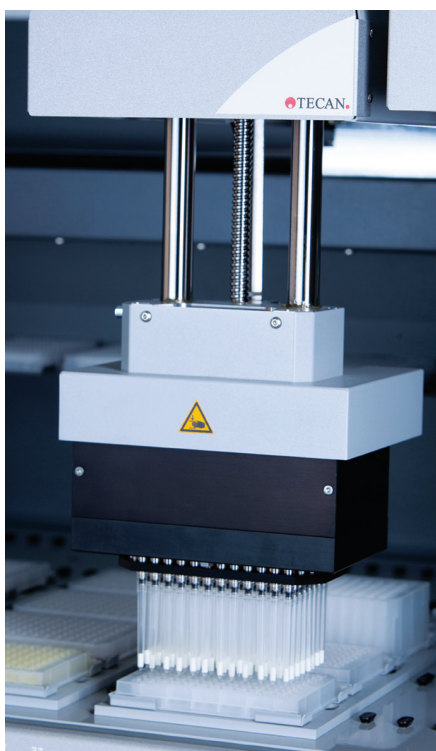
increases the rate of immobilization compared to shaking with bead-based supports, allowing the incubation time to be reduced – by up to 75 % in some cases – without affecting recovery rates. The capacity of these columns is also very good, allowing the capture of target molecules across a broad dynamic range.”

“As the microcolumns are housed within pipette tips, this approach is ideally suited to automation with a liquid handling platform. When you go down the automation route, there is obviously a significant investment involved, so you want to be able to do everything in much higher throughput. We have worked with a number of different liquid handling systems in the

past, but selected the Freedom EVO® workstation for the development of our latest product – MSIA Streptavidin EVO micro columns – due to the high capacity, robustness and flexibility this platform offers. In my mind, Tecan is a company that really pushes the boundaries of laboratory automation and system integration, and the Freedom EVO is very easy to configure to your exact workflow.”

“The microcolumns are designed specifically to work with the platform’s MultiChannel Arm™ (MCA) 96, allowing high throughput parallel processing of up to 96 samples in under two hours, but it is the workstation’s versatility that is the real advantage. You can integrate all

the necessary devices and labware onto the instrument’s large workdeck, providing completely walkaway sample preparation. As we continue to develop applications on the system, we have also been impressed by the versatility of the software – it has a lot of built-in functionality – and we hope to take advantage of this further in the future,” Eric concluded.



The microcolumns are designed specifically to work with the MCA 96 for optimal performance

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

To learn more about the Thermo Scientific MSIA Streptavidin EVO micro columns, go to www.thermoscientific.com/en/products/mass-spectrometric-immunoassay.html