

P-T-117 Protein Purification Utilizing a Solid-State UV Laser and Diode-Based Detector and Three Modes of Chromatography

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Introduction:

Chromatography is one of the commonly used strategies to isolate proteins and peptides for different levels of purities and concentrations. The various modes of liquid chromatography allow a wide range of flexible biomolecule separations. The different modes of separation rely on specific molecule interaction as the size or MW range of the target biomolecule, polarity/lipophilicity, ionic charge, affinity, or immunoaffinity.

In this example, a collaboration with a key customer to validate the KeyView solid-state UV detector for a protein purification separation. They utilized Size Exclusion Chromatography (SEC), Affinity, and Immobilized Metal Affinity Chromatography (IMAC) techniques to characterize a protein mixture of between 20 - 70 kDa with the KeyView UV detector.

This preparative separation and subsequent KeyView based detection became part of routine production of the target proteins. We are still evaluating KeyView's long-term lifetime and stability of the diode array and system. Under heavy use, the KeyView system has shown no change in performance.

Results:

Under SEC, IMAC, and Affinity conditions the KeyView was used to detect and trigger the fraction collection of the target protein. Under all conditions, the KeyView provided consistent and reliable detection under long usage. The KeyView channel of 278 nm was used in all three chromatographic conditions.

Conclusions:

The KeyView LED and Laser based UV detector provided reliable and consistent detection for the separation of a protein mixture across three common modes of preparative chromatography. The collaboration is ongoing as the detector has been incorporated for the routine production of this target protein. Further collaboration will help us better understand the long term lifetime of the KeyView detector systems

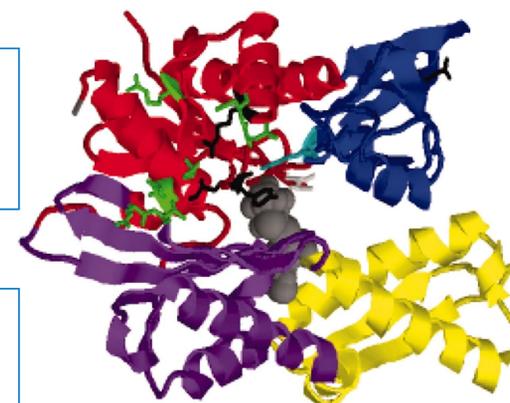


Figure 1
Representation of Protein Structure

Methods:

A collaboration with a key customer to validate the KeyView solid-state UV detector for a protein purification separation. The KeyView was tested under Size Exclusion Chromatography (SEC), Affinity, and Immobilized Metal Affinity Chromatography (IMAC) techniques to purify a protein mixture under preparative conditions. The application was run under gravity and peristaltic pump conditions. The target protein was between 20 - 70 kDa. Utilizing both the mobile phase conditions of tris(hydroxymethyl)aminomethane and a phosphate buffer. The LED based wavelength of 278 nm was used given the absorption spectrum of the target protein. The same wavelength and KeyView detector procedure were used across all three modes. The procedure has been adopted into routine use and long duty cycles.

Phoseon's KeyView™ detector utilizes patented SLM technology to improve both performance and ease of use over traditional light sources such as deuterium, tungsten, and xenon lamps. Phoseon Technology has overcome one of the critical obstacles for generating the 220 nm wavelength used in chromatographic applications by employing second harmonic generation using a 440 nm diode source. Using the refractive properties of a prism, the 440 nm fundamental wavelength produces a 220 nm high efficiency LED light source capable of replacing traditional UV lamps in chromatography applications.



Figure 2
KeyView™ UV Preparative Detector



Figure 3
Size Exclusion Chromatography Example - 278 nm.

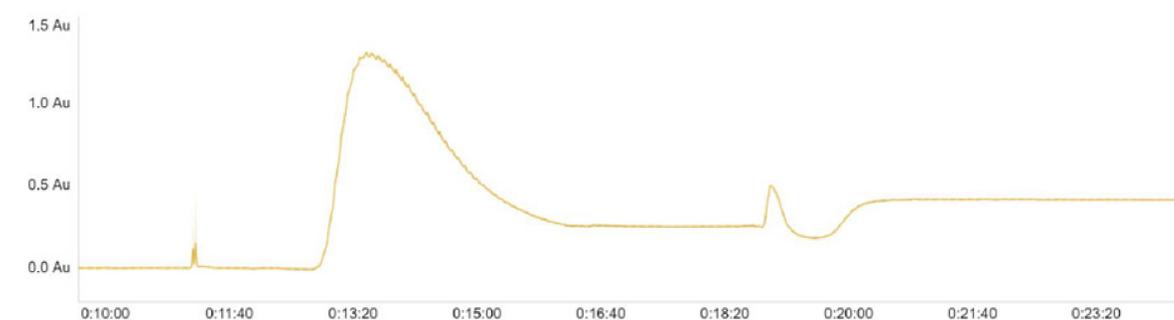


Figure 4
Immobilized Metal Affinity Chromatography - 278 nm.

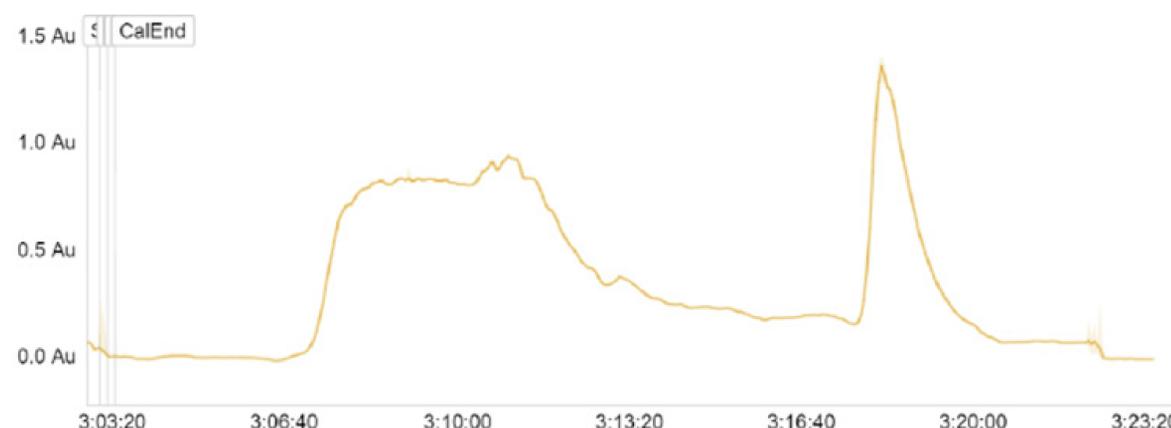


Figure 5
Affinity Chromatography - 278 nm.

References:

<https://phoseon.com/life-sciences/life-sciences-applications/#chromatography>



Further Questions?
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www.phoseon.com/life-sciences