



Wyatt Technology's Dynapro plate reader

Material compiled by
The Scott Partnership



nature@scottpr.com

www.scottpr.com

Pure and Simple!

The purification of proteins and peptides provides a vital first step towards understanding their function. Purification involves a series of processes that enables the isolation of a single type of protein or peptide from a complex mixture and is essential for the characterization of a significant protein. By attaining pure proteins or peptides, biochemists can determine amino acid sequences and evolutionary relationships between proteins in diverse organisms as well as investigate a protein's biochemical function. Furthermore, crystals of the protein may be grown from pure protein and from such crystals x-ray data can be obtained that will provide a picture of a protein or peptide's tertiary structure. Proteins and peptides can be purified in active form on the basis of solubility, size, charge or specific binding affinity and multiple methods can be used to achieve this. From gel electrophoresis to gel-filtration chromatography and thin layer chromatography to dynamic light scattering, a range of powerful purification techniques are available that will yield a sample containing the single protein that is of interest to the biochemist.

Pre-Purification

The use of pre-purification processes allow for the rapid and efficient separation of a protein or peptide of interest from interfering compounds and dramatically increases the yield and purity of a sample for subsequent purification steps. **Millipore** has introduced the new **Amicon Ultra-0.5 mL** filter, the newest in the Amicon Ultra line of centrifugal filters for protein concentration and buffer exchange. Offering 25- to 30-fold concentration with 90% recovery rates in as little as ten minutes of centrifugation, the Amicon Ultra-0.5 mL device provides the highest recovery with the fastest spin times in its product class. The device is currently available with either 3,000 or 10,000 molecular weight cut-off (MWCO) ultrafiltration membrane. Quality sample concentration is a critical step in protein biochemistry as it is not only the final stage of a lengthy purification process, but it is also the step that determines the success and reproducibility of downstream analyses. Structural biologists are highly dependent on quality sample concentration and routinely require extremely concentrated samples, up to 20 mg/mL. This is made possible by the high recovery achieved with Amicon Ultra filters.

Dynamic Light Scattering

Dynamic Light Scattering (DLS) processes the time-dependent fluctuations in scattered light intensity to yield the hydrodynamic radius of proteins in a solution.

Wyatt Technology's Dynapro plate reader DLS instrument enables the rapid detection and characterization of protein aggregates. Many of the Dynapro's users are scientists involved in the research and development of drugs or vaccines that are made from proteins. It is essential that these scientists are able to ensure that their proteins and

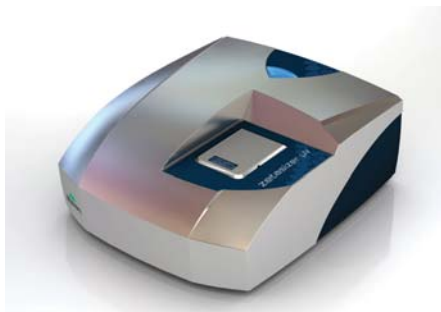
protein-based particles are the correct size and remain the correct size over time and throughout exposure to a variety of processes and conditions. The Dynapro plate reader measures the protein size in hundreds of different samples automatically, enabling scientists to perform critical measurements in less time with less "hands-on" attention. According to the company, Dynapro technology is the only technique in the world that can measure the sizes of protein samples contained in microplates containers that can hold thousands of samples without perturbing them. In doing so, the Dynapro plate reader utilizes less precious protein, provides higher degrees of reproducibility and measures samples more quickly and easily when compared to traditional instruments.

The **Zetasizer** from **Malvern Instruments** is an advanced light scattering system. Designed for protein specialists it is dedicated to providing improved understanding of protein applications, including: optimising storage conditions; verifying the quaternary structure of protein complexes; identifying thermal characteristics; assessing protein solubility and sample purity.

High-Pressure Liquid Chromatography

High-pressure Liquid Chromatography (HPLC) is a form of column chromatography used to separate, identify and quantify peptides and proteins based on their idiosyncratic particles and interaction with the column's stationary phase.

GE Healthcare has announced the launch of **ÄKTA™** avant, the next generation ÄKTA high performance liquid chromatography system providing a complete, robust solution for fast, scalable and high quality protein separations in process development. The built-in UNICORN™ 6 control system with integrated Design of Experiments



The Zetasizer IV from Malvern Instruments

(DoE) functionality saves time and increases productivity. The advanced configuration, flow and pressure capabilities of AKTA avant enable the use of modern, high-flow BioProcess chromatography media including MabSelect™ and Capto™, which can shorten process times by days and provide significant cost savings.

Thin-Layer Chromatography

The lack of an efficient, cost-effective technique to purify blocked peptides has been problematic in peptide synthesis. Fully protected peptide segments are bulky, very hydrophobic and are often difficult to purify. Thin-Layer Chromatography (TLC) offers a fast and versatile technique used to develop and optimize separations for FLASH chromatography. The **Biotage FLASH 12** system provides a new powerful tool to efficiently and easily isolate the protected peptide segments resulting in high purity and good mass recovery. The system overcomes the problems encountered in traditional gravity column chromatography including low resolution, long separation time, inferior sample recovery and large solvent and adsorbent consumption.

Purification of Tagged Proteins

Adding a tag to a protein gives it a binding affinity that it would not otherwise have. Usually the recombinant protein is the only protein in the mixture with this affinity, which aids in separation.

The new **Promega HaloTag® Protein Purification System** addresses common problems associated with purification of recombinant proteins by enabling the delivery of soluble, functional recombinant target proteins at higher yield and purity. The HaloTag protein purification system consistently delivers soluble protein and outperforms the most commonly used protein tags like His (6)Tag, GST and MBP. Beta testers credited the HaloTag protein purification system as an exciting alternative for researchers working with difficult-to-express proteins. Specifically, testers noted that the simple, fast detection and ease-of-use of the HaloTag system solved many of the problems encountered with other expression methods that lead to low or no yield and poor purity. The HaloTag technology is based on a unique protein tag which has been engineered from a bacterial dehalogenase to covalently attach to a set of synthetic ligands, such as fluorescent dyes, for cellular imaging applications or to resin and magnetic beads for protein interaction analysis applications.



The Promega HaloTag® protein purification system

Protein Purification Reagents

Activated affinity support products are available to immobilize nearly any type of ligand to purify its binding partner(s).

Thermo Fisher Scientific has launched a new range of protein purification and preparation products including the second generation of the popular **Thermo Scientific Slide-A-Lyzer Dialysis** product line. The newly designed and patented Slide-A-Lyzer® G2 Dialysis Cassettes offer sample loading and removal with a pipette or hypodermic needle. The new cassettes have built-in air chambers for buoyancy and vertical orientation, eliminating the need for separate floats. The Slide-A-Lyzer G2 line offers units with sample capacities of 100 microliters to 70 milliliters.

Protein Expression

The expression of a protein from a gene sequence is controlled by numerous factors. Some of these factors are defined in the untranslated regions of the gene (e.g., the promoter regions) or within the gene sequence (e.g., intron/exon splice regions). The cell or tissue type also has effects on the expression of specific proteins.

Thermo Fisher Scientific has introduced an innovative in vitro protein expression system that improves protein production and post-translational modifications of proteins. The Thermo Scientific Pierce In Vitro Glycoprotein Expression Kit synthesizes and glycosylates protein in vitro at much higher efficiencies than current commercially available methods. The easy-to-use protein synthesis and glycosylation reaction requires only 90 minutes, without the addition of other components such as microsomal membranes. When protein glycosylation is not necessary, the Pierce In Vitro Protein Expression Kit is available. Both kits rely on novel human cell lysates for the necessary protein synthesis machinery. The brief 90-minute reaction produces up to four-fold higher protein expression over commonly used in vitro translation systems. In addition, the Pierce In Vitro Expression Kits continue to synthesize proteins for up to six hours, resulting in much higher yields.

Ion-Exchange Chromatography

Proteins can be separated on the basis of the nature and degree of their ionic charge by ion-exchange chromatography.

Agilent Technologies has introduced new BioHPLC columns specifically designed for the analysis of bio-molecules including monoclonal antibodies, recombinant proteins, peptides,



The Thermo Scientific NanoDrop 2000c offers easy, reliable microvolume analysis (1uL)

vaccine products and DNA/RNA. The columns provide robust, reproducible and high-resolution solutions for biopharmaceutical manufacturers to effectively monitor the safety, efficacy and stability of products. These new bio-columns offer customers significant improvement in charge-based and size-based analytical separations of bio-molecules. Each new column is individually tested with protein samples to ensure column-to-column and batch-to-batch reproducibility. The four new column families include: **Agilent Bio SEC-3** size exclusion columns, packed with 3µm particle, which offers higher resolution and faster separation times and **Agilent Bio SEC-5** size exclusion columns, packed with 5µm particles coated with neutral, hydrophilic coating, making the particle extremely stable under harsh buffer conditions.

Post-Purification

Once purified, it is essential that biochemists measure the purity of the protein or peptides they have processed. Ensuring this process is efficient while maintaining accuracy is essential. **Thermo Fisher Scientific** has introduced the **Thermo Scientific NanoDrop™ 2000** and **2000c** spectrophotometers that require only 2µL of protein sample for highly accurate quantitation without the need for cuvettes and capillaries. The user simply pipettes a 2µL sample directly onto a fiber optic measurement surface where it is held in place by a patented retention system. During each measurement cycle, the full absorbance spectrum of the sample is assessed at four different path lengths (1.0 mm, 0.2 mm, 0.1 mm and 0.05 mm) which is automatically optimized for each sample. The path length optimization results in an extensive dynamic range of measurable concentrations (0.01 mg/mL to 400 mg/mL for BSA), virtually eliminating the need for dilutions.

Companies featured in this product focus:
 Millipore Corporation - www.millipore.com
 Wyatt Technology - www.wyatt.com
 Malvern Instruments - www.malvern.com
 GE Healthcare - www.gehealthcare.com
 Biotage - www.biotage.com
 Promega - www.promega.com
 Thermo Fisher Scientific - www.thermoscientific.com
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