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Automate Your Protein Sample Preparation

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Executive Summary

Working with proteins is challenging. Often protein analysis, in-gel digestion for mass spectrometry or protein purification are done manually. But at Alphalyse® in Odense, Denmark, the lab team established their protein analysis on the automated liquid handler ep*Motion*® 5075vt to increase reproducibility, quality of results and free up time for other tasks. Automation can be beneficial, not only for protein work. Also, DNA and RNA applications can be improved, simplified and optimized. Read more about the work of Alphalyse and the benefits of automating daily lab processes.

Introduction

Proteins are the most versatile and manifold macromolecules in nature. Understanding their functions, molecular structures, or their interactions with other molecules are the aim and the objectives of thousands of scientific publications to date. However, it is obvious that we are still at the very beginning of understanding these amazing molecules that are the fundament of living cells and so of the whole biosphere. Our motivation to learn more about these 'sensitive lab pets' is substantiated by the great biomedical, biotechnological and biomechanical use of these insights.

But everybody who is working with proteins in the lab knows: The sample preparation for all those numerous techniques to analyse proteins or peptides is very, very laborious. Not only that it takes a lot of time to properly process the samples, but each protein wants to be prepared and treated in a very certain way. Therefore, it is not uncommon that "protein people" end up with high numbers of protocols and workflows, a lot more than DNA or RNA people usually use.

Thus, one can say that the preparation of samples for protein analysis is on the one hand tedious and time consuming, and on the other hand exceptionally complex and variable. Getting back to the title of this white paper you might wonder how any automated sample preparation can ever be able to cope with such challenging requirements.

A protein lab that employs an automation platform for sample preparation

Alphalyse, a contract research organization in Odense (Denmark) and Palo Alto (USA), is an established partner of the biotech industry, pharmaceutical companies and academic research groups worldwide for protein analysis and characterization services. The laboratory team at Alphalyse deals with a large variety of sample types (biosimilars, antibodies, peptides, vaccines, etc.), and copes with a lot of different analysis workflows such as host cell protein analysis and cutting-edge mass spectrometry techniques.

Alphalyse recently acquired a new automated liquid handling system from Eppendorf, the ep*Motion* 5075vt. They named this liquid handler Pippi, as this robust system has already become a valued and reliable member of the team. Pippi performs many different sample preparation protocols freeing up time for staff for planning experiments and discussing the results. In the following paragraphs they describe their experiences when using the ep*Motion* 5075vt for their protein sample preparation.

Characterization of proteins – sequencing, disulfide bridges, glycosylations, etc.

At Alphalyse the liquid handling platform ep*Motion* 5075vt (Pippi) performs a complete workflow starting from soluble protein, to digestion and protein purification. Their in-house protocol employs two different SPE filter plates, which can be processed on Pippi´s vacuum module. Protein digestion is performed on the integrated Eppendorf ThermoMixer® position (4-90 °C, up to 2,000 rpm for up to 24 hours) while samples are automatically transported by a gripper. Throughout the complete process of up to 8 hours there is only two user inventions to rearrange the labware on the liquid handler deck. This protocol can be performed with 1 to 96 samples which allows for a high flexibility on a daily basis freeing up time for other tasks.

In-gel digestion of proteins

The preparation of proteins that have been separated in an SDS-PAGE is a standard method for “protein people”, and so at Alphalyse. Their in-house protocol starts with tiny pieces of gel material containing the desired proteins in a 96 well microtiter plate. The whole process starts with dehydrating, reducing and alkylating the gel material, followed by protein digestion using specific proteases. The digestion reaction is terminated by adding TFA. Pippi even takes over the loading of the mass spectrometry plate (MTP AnchorChip™ 384), which requires an experienced and steady hand if done manually.

Useful functions and abilities of the ep*Motion* 5075vt for "protein people"

a. Allrounder for every liquid handling task
In each laboratory, there are pipetting tasks to be done that do not need a lot of knowledge but are very tedious. Just think about preparing serial dilutions, reformatting samples from tubes to plates, aliquoting enzymes, standards or samples, cherry-picking individual samples out of plates, normalize the protein concentration in a sample set, filling plates with medium, etc. Thinking of your daily or weekly tasks there will be even more that could be automated. Employing a liquid handler for everyday tasks will free up your hands and mind for experiment planning and analysing results. The ep*Motion* is also an expert in handling magnetic beads, any (immune) assays, handling large stacks of plates and many more. You will be surprised by its versatility and how easy it is to increase your sample throughput and streamline your daily tasks.

b. Day-by-day performance of variable processes
It is very common for protein preparation procedures to be very variable. The time and temperature for protein digestion steps for instance, as well as the numbers of sample, washing cycles and added volumes can vary a lot between to processing batches. You might think that such high variability is less ideal for automation, but that is not the case. When using the ep*Motion* 5075vt it is easy to accomplish for variable parameters in a method that serves as master for multiple sample types and proteases. To begin with, the epBlue™ software allows methods to be run with variable numbers of samples. Shaking or tempering parameters as well as the incubation time can be adjusted for each run individually. With the epBlue user and ID management system and its logfile documentation you will not lose track of any sample or method which has been run by any user. The run logfile serves as a comprehensive output file of the sample process that has taken place on the ep*Motion*.

c. Enhance your reproducibility
Automated liquid handlers are the best investment in a lab can do to enhance the reproducibility of experimental results, and to increase the sample throughput. Once established on the ep*Motion* 5075vt, a protocol will perform reliably and robustly throughout and at any time. Aside of a higher reproducibility when using liquid handlers, you will also save a lot of time and reagents just because you do not have to repeat experiments so frequently. When using an automated liquid handler you will be able to accomplish reproducibility without relying on just one single person.

d. Resource-friendly processing
The platform works very consumable sensitive.
Firstly, protein purification protocols count on filter plates where proteins or peptides can be washed multiple times. The ep*Motion* allows tips to be re-stored and re-used if the same liquid is transferred. This considerably reduces the plastic waste associated with sample preparation, while the accuracy and precision of the liquid transfer is not impaired. Secondly, the platform can be programmed in a way that each and every well of a 96-well filter plate can be used in independent sample processing runs. So, even if it requires a bit of exercise and concentration to tell the system which specific wells are the ones for today´s run, it is possible to be as cost sensitive with expensive filter plates as you used to be in the manual processing.

e. Minimize the protein contamination during the process
There are some proteins that are highly abundant in the environment and can therefore reduce the sensitivity of analysis for the protein of interest. Aside from keeping a dust-free environment in their labs, Alphalyse reduces the impact of the unloved "keratin rain" by keeping the housing of the system shut during the whole process. In addition, the ep*Motion* covers plates with lids automatically during incubation periods. Further, the surface of the ep*Motion* 5075vt can be wiped with ethanol and soft detergents to reduce the present protein contamination in the system. All accessories as well as the pipetting tools can be autoclaved in addition.

Conclusion

Working with proteins requires a lot of lab experience because protocols can be both complex and highly variable. Robust and reliable liquid handling systems like the ep*Motion* 5075vt with its integrated Eppendorf ThermoMixer and vacuum manifold are perfect assets for protein laboratories that free-up time for experiment planning and data analysis. Like their customers in research, biotech and pharma, Alphalyse strongly relies on the reproducibility of results. Acquiring the ep*Motion* 5075vt liquid handler from Eppendorf increased the sample throughput as well as the overall performance of their protein analysis services.

More about Alphalyse
Alphalyse is a contract research company (CRO). They possess cutting-edge expertise in mass spectrometry-based protein analysis for research, production and clinical development of native and recombinant proteins. Through standard protein analysis services and customized analytical services, the company offers a range of unique high-quality protein analyses to identify and characterize biopharmaceutical proteins. Examples also include monoclonal antibodies and vaccines. The services are used by the biotech industry, pharmaceutical companies and academic research groups worldwide. Alphalyse has offices in Odense, Denmark and Palo Alto, California, USA.
www.alphalyse.com

About Eppendorf

Since 1945, the Eppendorf brand has been synonymous with customer-oriented processes and innovative products, such as laboratory devices and consumables for liquid handling, cell handling and sample handling. Today, Eppendorf and its more than 3,000 employees serve as experts and advisors, using their unique knowledge and experience to support laboratories and research institutions around the world. The foundation of the company's expertise is its focus on its customers. Eppendorf's exchange of ideas with its customers results in comprehensive solutions that in turn become industry standards. Eppendorf will continue on this path in the future, true to the standard set by the company's founders: that of sustainably improving people's living conditions.

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