

proteomics

¹⁸O Proteome Profiler Kit: A Useful Tool for Relative Quantitation of Global Differential Protein Expression

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Application Notes

- Convenience – the product is designed with prepackaged one-time use reagents
- Complete kit with all of the reagents necessary for labeling
- Incorporation of the isotopic label allows for easy determination of relative protein amounts
- Less expensive than other isotopic labeling methods, such as ICAT or AQUA

Introduction

Differential protein expression is an area of great interest in proteomics. Isotopic labeling is a commonly used method for relative protein quantitation. Unfortunately, isotopic labels are often very expensive and difficult to work with. The ¹⁸O Proteome Profiler Kit (Product Code [P 3623](#)) eliminates these problems by providing a cost-effective and easy method for performing relative protein quantitation. The basis of the kit is the enzymatic incorporation of a stable isotope label (¹⁸O) into virtually all peptides in a tryptic digest. Because oxygen atoms do not rapidly exchange in the absence of the protease, this method is highly specific for stable isotope incorporation into the carboxyl terminus of each new peptide. This method is considered to be a global labeling procedure since only the protein's C-terminal peptide will not have a stable isotope incorporated (unless the C-terminal amino acid is an arginine or lysine). The incorporation of two ¹⁸O atoms onto each peptide results in a 4 Dalton mass shift, which is readily observed when the samples are analyzed by Mass Spectrometry (MS). Analysis of the mass shift allows labeled and unlabeled samples to be differentiated, and their relative amounts quantified.

Labeling method

The ¹⁸O Proteome Profiler Kit is designed to allow for the comparison of two protein populations. Prior to using the kit, the samples should be denatured, reduced and alkylated. Next the detergents, chaotropes, and reduction and alkylation reagents are removed using the spin columns provided with the kit. The proteins are subsequently digested, and the enzymatic activity of trypsin is used to incorporate the ¹⁸O label into the tryptic peptides. Trypsin is capable of incorporating two ¹⁸O atoms per peptide (Figure 1). This is due to the nature of the extended interaction between peptide and protease, allowing for equilibration of both carboxyl oxygen atoms

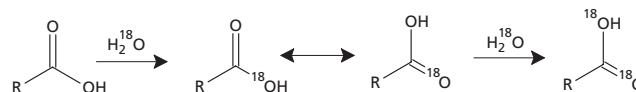


Figure 1. Trypsin catalyzed incorporation of ¹⁸O into both carboxyl oxygen atoms of the C-terminal amino acid.

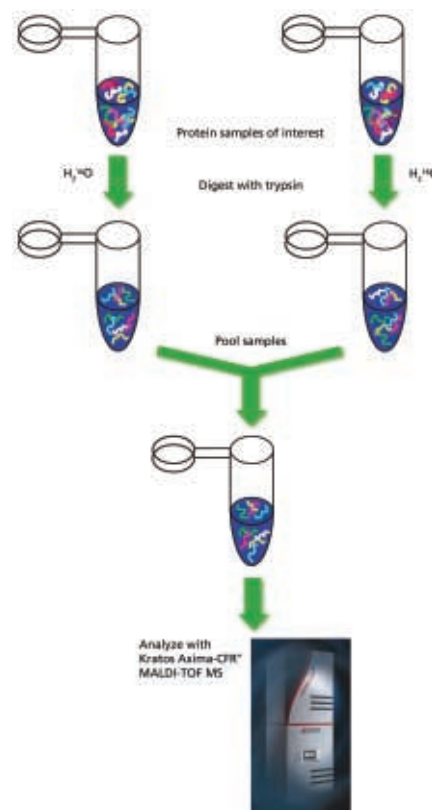


Figure 2. Illustration of the workflow. Two sample populations are prepared in parallel up to the point of labeling. Following incorporation of the label (either ¹⁶O or ¹⁸O), the two samples are mixed and analyzed by mass spectrometry.

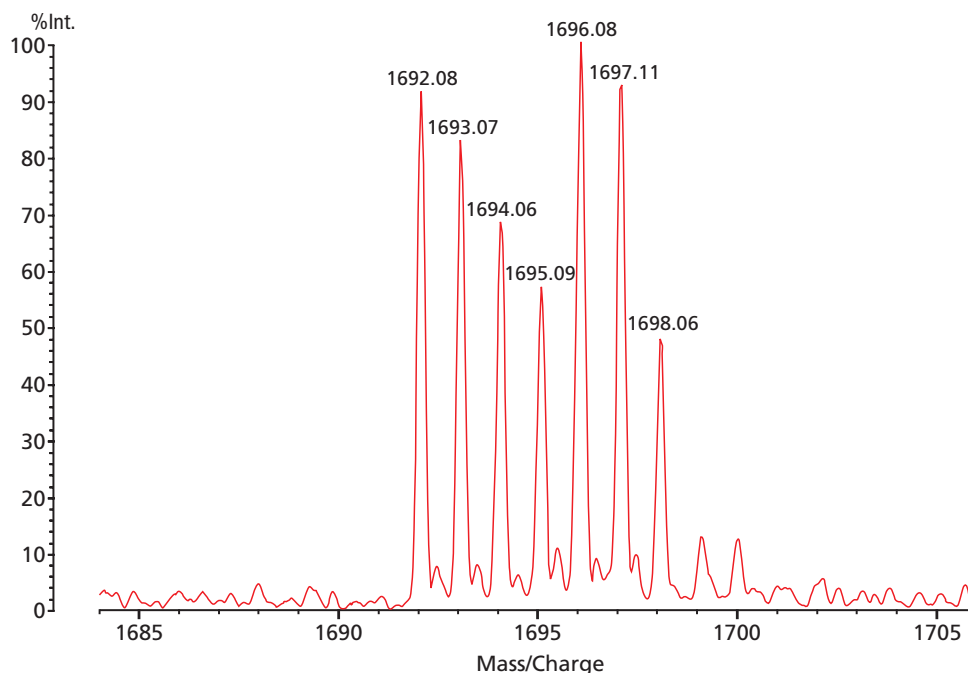


Figure 3. MALDI-TOF mass spectrum of the peptide YSHEEIAMATVTALR derived from Aldolase. This represents expected results obtained when two protein solutions of equal concentration are analyzed using this kit. The base peak at m/z 1692.08 (mass + 0 Da) has approximately the same intensity (I_b) or integrated peak area as the peak at m/z 1696.08 (mass + 4 Da, I_d). The peaks at 1693.07, 1694.06, and 1695.09, (as well as those at 1697.11 and 1698.06) represent the normal isotopic distribution obtained from the mass spectrometric analysis of a peptide.

at the C-terminus. Because two ^{18}O atoms will be incorporated, the labeled peptide is shifted by + 4 Daltons when viewed on a mass spectrum. The samples are then analyzed via an appropriate method such as MALDI-TOF MS or LC-MS. The workflow is illustrated in Figure 2.

The utility of the ^{18}O Proteome Profiler Kit was demonstrated by evaluating two different protein samples. For this particular experiment, the protein samples consisted of a mixture of Carbonic Anhydrase (Product Code [C 4396](#)) and Aldolase (Product Code [A 2714](#)) at known concentrations. The samples were denatured in 8 M Guanidine HCl, buffered to pH 8.5 (Product Code [G 7294](#)) and then reduced and alkylated using the ProteoPrep Reduction and Alkylation kit (PROT-RA). The samples were then desalted and tryptically digested using the reagents provided in the ^{18}O Proteome Profiler Kit. Following digestion, both samples were vacuum dried. Using the provided Trypsin Singles (Product Code [T 7575](#)), the label (either ^{16}O or ^{18}O) was incorporated into the test and control samples. The samples were allowed to incubate overnight at 37 °C, vacuum dried the next day, reconstituted with 0.1% TFA, mixed and analyzed by MALDI-TOF MS. Analysis of the data showed that the observed ratios for both proteins were accurate to within 25% of their theoretical concentrations (Figure 3).

Summary

The ^{18}O Proteome Profiler Kit serves as a reliable method for isotopic labeling of protein samples. The kit contains all of the reagents necessary for labeling, and is considerably cheaper than other comparable methods such as ICAT or AQUA. Use of the ^{18}O Proteome Profiler Kit, provides a simple and effective method for incorporation of an isotopic label, and subsequent analysis of differential protein expression.

Coming in Summer 2005!