

proteomics

Targeted Gene Knockdown Experiments: Combining ^{18}O -Isotopic Labeling and Protein-AQUA™

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Introduction

Targeted gene knockdown using RNA interference (RNAi) has proven to be a powerful tool for studying gene function in eukaryotes. Methods currently employed for studying samples at the protein level, while sensitive, often lack the ability to discriminate between samples in which the differences in protein levels are minimal. Additionally, the data generated through this existing methodology is semi-quantitative at best. In the present work, we present a quantitative proteomics approach for the analysis of gene knockdown experiments, through the combination of ^{18}O -isotopic labeling and Protein-AQUA™.

Easy, Cost-effective Protein Quantitation

The analysis of protein expression is an area of great interest in proteomics. Isotopic labeling is commonly employed in a variety of relative protein quantitation methods. Unfortunately, currently available isotopic labeling methods are often very expensive and difficult to utilize.

In June 2003, Dr. Steve Gygi and his team at Harvard presented a strategy for absolute protein quantitation by employing isotopically labeled peptides for downstream analysis by LC-MS/MS. This technique, termed Protein-AQUA, is based on a common principle – the use of an isotopically labeled internal standard. By applying this principle to the quantitation of proteins, Gygi's team has advanced the ability of protein researchers to study complex biological samples quantitatively.

The first step of the Protein-AQUA approach (Figure 1) involves the selection of a peptide from the protein of interest. Next, an isotopically labeled analog of the selected peptide is synthesized. Following extraction of a biological sample, a known quantity of the AQUA peptide is added. The Protein-AQUA sample is proteolytically digested and analyzed by mass spectrometry. By comparing the native peptide signal to the AQUA Peptide signal, an absolute amount of the protein of interest can be calculated. The Protein-AQUA methodology provides a targeted proteomics approach that allows for absolute quantitation of a single protein in a complex mixture.

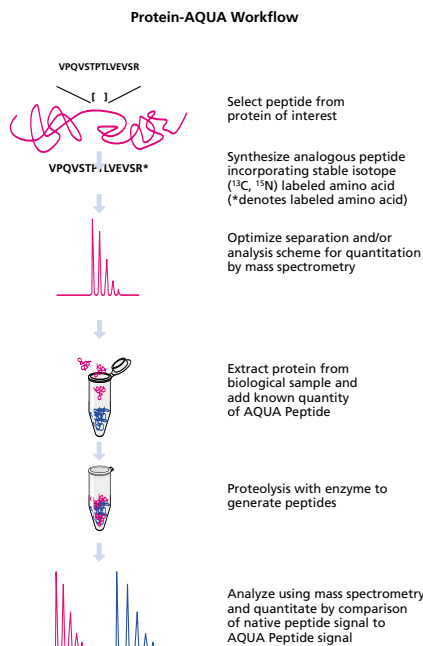


Figure 1. This method was developed by Dr. Steve Gygi and colleagues at Harvard Medical School [Stemmann O, Zou H, Gerber SA, Kirschner MW. Dual inhibition of sister chromatid separation at metaphase. *Cell* 2001, Dec 14, 107, 715-726]. Limited use of this method is permitted under a licensing agreement with Harvard Medical School.

While the Protein-AQUA approach allows for focused analysis, global labeling techniques offer a variety of advantages. The ^{18}O Proteome Profiler Kit (Product Code P3623) provides a cost-effective and facile method for performing relative protein quantitation. The kit allows for the comparison of two protein populations, typically a "test" sample and a "control" sample. The basis of the kit is the enzymatic incorporation of a stable isotope label (^{18}O) into virtually all peptides in a tryptic digest. As outlined in Figure 2, the ^{18}O labeling method begins with proteolytic digestion of the two samples. Next, the global incorporation of either ^{16}O or ^{18}O is carried out through incubation with the appropriate solvent and trypsin. The samples are combined in a 1:1 ratio, and analyzed by mass spectrometry. For each peptide in the sample, the signal from the labeled (^{18}O) and unlabeled (^{16}O) samples can be compared. This methodology allows for differential protein expression analysis between the two samples. Most importantly, this method is considered to be a global labeling procedure, since only the protein's C-terminal peptide will not have a stable isotope label incorporated (unless the C-terminal amino acid is an arginine or lysine).

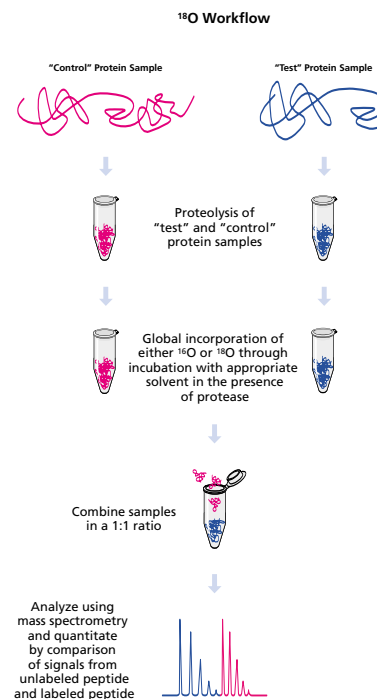


Figure 2. Overview of ^{18}O -labeling workflow.

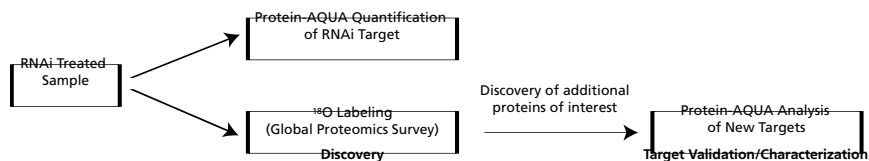


Figure 3. Proposed ^{18}O /AQUA Sequential Protein Quantitation Workflow.

Summary

When used in combination, ^{18}O enzymatic labeling and Protein-AQUA provide a synergistic and efficient approach for the analysis of gene knockdown experiments. Both techniques offer exquisite sensitivity by employing mass spectrometry for downstream analysis. As outlined in Figure 3, the first step in this proposed method would employ both ^{18}O labeling and directed Protein-AQUA analysis of the sample. Specifically, the Protein-AQUA analysis would enable absolute quantitation of the protein targeted for gene knockdown. Because the ^{18}O method is a global labeling procedure, it allows for the elucidation of off-target effects, as well as the identification of related protein products affected by the knockdown event. Subsequent to this initial survey, a more targeted approach can then be employed using the Protein-AQUA technique for further detailed analysis of the newly identified protein(s) of interest. By sequentially exploiting the unique features of each technology, this proposed strategy would enable protein researchers to investigate RNAi experiments in a way previously unavailable. Additionally, because the analysis of gene knockdown is accomplished at the protein level, this technique allows for true functional validation of RNAi experimentation.

To learn more about AQUA Peptides, visit our Web site at sigma-aldrich.com/aqua

Protein-AQUA is a trademark of Harvard University.

Ordering Information

Product	Description	Unit
	Custom AQUA Peptide (quantitated by AAA)	1 x 5 x 1 nmol 10 x 5 x 1 nmol
	Custom AQUA Peptide (quantitated by fluorescamine)	1 x 5 x 1 nmol 10 x 5 x 1 nmol
P3620	^{18}O Proteome Profiler Kit*	1 kit

*Available December 2005