

Agilent Proteomics Reagents for LC/MS

Product Note

For sample preparation in proteomics applications such as biomarker discovery, protein characterization, and shotgun proteomics, Agilent offers Proteomics Grade Trypsin, FFPE Protein Extraction Solution, and PPS Silent Surfactant. These reagents are directly compatible for use with LC/MS methods and do not require any additional sample preparation or purifications.



Agilent Technologies

Proteomics Grade Trypsin

- High specificity and purity to prevent autolysis and ensure consistent performance
- Qualified with Agilent HPLC-Chip and 6000 Series LC/MS systems to ensure accurate results
- Protocols for in-solution and in-gel digestion to provide flexibility for different applications

High specificity and purity

Agilent Proteomics Grade Trypsin is a highly pure serine protease that specifically cleaves the carboxylic side of lysine and arginine residues without generating autolytic fragments. To ensure high purity and prevent the generation of autolytic trypsin fragments (which is undesirable and can complicate mass spectrometry analysis of unknown peptides), the porcine pancreas trypsin is reductively methylated to produce an enzyme that retains activity on exogenous substrates but is highly resistant to autolysis. The modified enzyme preparation is then treated with TCPK, an inhibitor of the common contaminant chymotrypsin, to fully prevent generation of autolytic peptide fragments. Finally the modified, treated trypsin is affinity purified and lyophilized to ensure maximum purity and stability.

Ideal for MS analysis

Each lot of Agilent Proteomics Grade Trypsin is tested and qualified on the Agilent 1200 HPLC-Chip technology combined with the Agilent 6000 Series LC/MS system (Figure 1). Qualification of each lot by functional testing in an actual LC/MS experiment ensures consistent performance and accurate results. To provide improved ease of use for multiple applications protocols are provided for both in-solution and in-gel digestion.

FFPE Protein Extraction Solution

- Detergent-free protein extraction, ideal for direct analysis by LC/MS
- Preservation of immunological epitopes for analysis by immunoassays, with increased sensitivity of detection
- Flexible for small and large sample sizes with reproducible extraction of full-length proteins and short polypeptides

Detergent-free extraction and preservation of immunological epitopes

Agilent FFPE Protein Extraction Solution is a detergent-free method for isolating proteins directly from FFPE tissue. Unlike other extraction buffers the Agilent FFPE Protein Extraction Solution

maintains protein epitopes for validation and confirmation of data by immunoassay (Figure 2). Since the method is detergent-free it yields full-length protein and short peptides that can be directly analyzed by LC/MS. This extraction solution is ideal for protein biomarker discovery and validation going from LC/MS analysis to immunoassays. Competitor products require precipitation steps that can lead to sample losses prior to MS.

Ideal for mass spectrometry and immunoassay

Extracting proteins from FFPE tissue is challenging and the use of detergents typically damages immunological

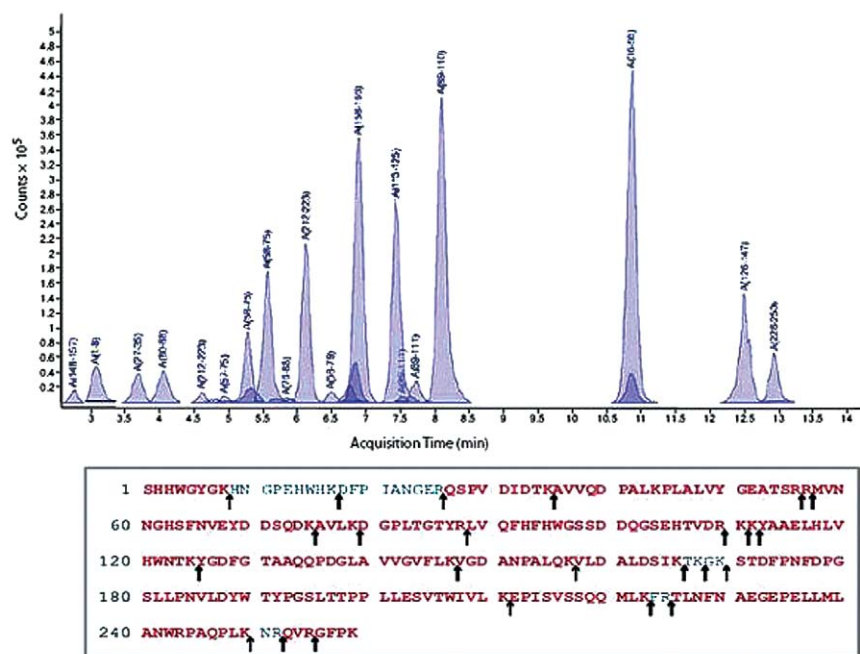


Figure 1. Total ion chromatogram and peptide coverage map of bovine carbonic anhydrase II digested with Agilent Proteomics Grade Trypsin and analyzed with the Agilent 6520 Accurate-Mass Q-TOF and the HPLC-Chip interface.

epitopes and complicates downstream analysis by proteomic methods. However, millions of FFPE samples are prepared each year and could be utilized for biomarker discovery and validation. The Agilent FFPE Protein Extraction Solution effectively extracts and relaxes proteins, yielding intact proteins with preserved immunological epitopes. Since the extraction solution is detergent-free the samples are ready for analysis by mass spectrometry.

PPS Silent Surfactant

- Solubilizes hydrophobic proteins to yield greater recovery of total proteins from a complex matrix
- Facilitates complete digestion of more proteins, yielding fewer nontryptic peptides
- Acid cleavable reagent does not interfere with MS identification of low abundance or low molecular weight peptides

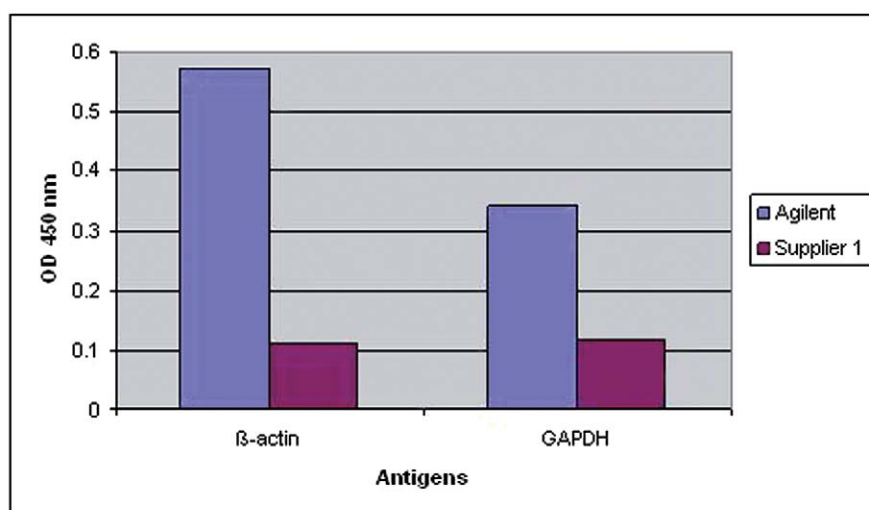


Figure 2. High Epitope Conservation and Recognition of β -actin and GAPDH. For each extraction, one section of FFPE normal liver tissue with an area of 1 cm² and a thickness of 10 μ m was extracted by either Agilent FFPE Protein Extraction Solution or a competitor's FFPE reagent. 1 μ g of each extract was used to coat a well of the 96-well plate. The expression of β -actin and GAPDH was detected by ELISA.

Improved analysis of complex protein mixtures

Agilent PPS Silent Surfactant is a mass spectrometry (MS) compatible detergent designed for the extraction and solubilization of hydrophobic proteins, the improvement of in-solution enzymatic protein digestions (e.g., trypsinization), and the disruption of cell membranes—ultimately increasing the number of total peptides and proteins identified in a sample, with increased coverage for a more comprehensive profile. Unlike other detergents used in MS analysis, PPS surfactant disrupts cell membranes to facilitate recovery of membrane proteins and through acid cleavage does not interfere with MS analysis. The properties of PPS surfactant make it suitable for improved analysis of complex protein mixtures by increasing the total number of proteins observed by MS analysis.

Acid-cleavable for enhanced identification

PPS Silent Surfactant is acid-cleavable so reducing the pH of the digestion buffer cleaves the reagent. This property of PPS surfactant avoids interference with MS readings and thus enhances the ability to identify low abundance peptides. As a result, PPS Silent Surfactant is ideal for shotgun proteomics, identification of low abundance proteins from a complex mixture, and for analysis of membrane proteins.

Custom configurations for large volume requirements

Our stringent procedures and expertise in manufacturing allow us to offer custom packaging configurations for large volume requests. For more information please visit <http://www.stratagene.com/custom>.

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