



Pressure-Enhanced Enzymatic Proteolysis

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Pressure BioSciences, Inc.

Pressure BioSciences, Inc. (NASDAQ: PBI) is a life sciences company focused on the development and commercialization of a novel, enabling platform technology called Pressure Cycling Technology ("PCT").

PCT Sample Preparation System

The Pressure Cycling Technology Sample Preparation System ("PCT SPS") employs rapid cycles of hydrostatic pressure, between ambient and ultra high levels, to precisely control biomolecular interactions. The PCT SPS can be used to accelerate enzymatic reactions, such as protein digestion with trypsin and other proteolytic enzymes, to prepare samples for analysis by mass spectrometry. In addition, the PCT SPS can be used to disrupt tissues and cells to extract cellular structures and components such as proteins, lipids, nucleic acids, and small molecules for further analysis. [1]. The PCT SPS is comprised of a Barocycler (a small, semi-automated bench-top instrument used to generate high hydrostatic pressure) and specially-designed, high pressure tolerant, single-use sample preparation tubes by an external circulating water bath, which can be an important addition to the Pressure Cycling Technology Sample Preparation System.

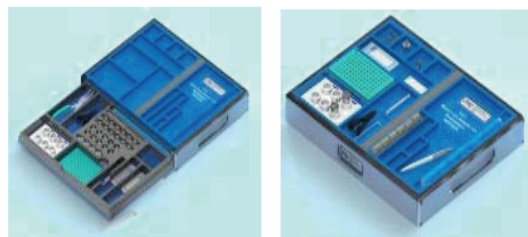
Barocycler



PCT MicroTube* Adapter Kit

Processing time, sample throughput, accuracy, efficiency, and standardization are key elements of the sample preparation process for mass spectrometry analysis. The PCT MicroTube Adapter Kit, in combination with the PCT SPS, can reliably and reproducibly control the enzymatic digestion of proteins while reducing the time of digestion from hours to minutes with the same or better quality as other, currently available techniques. The PCT MicroTube Adapter Kit comes complete with an ergonomically designed, space-saving Work Station containing PCT MicroTubes and PCT MicroCaps, as well as tools and hardware, to enable the user to process from one to forty eight samples simultaneously in the PCT SPS.

*Patent pending



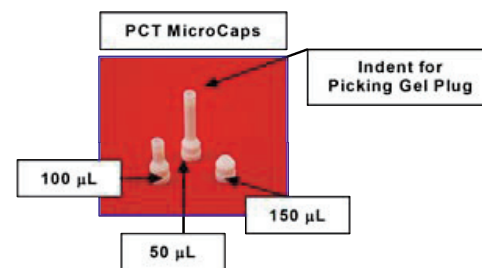
PCT MicroTube

The PCT MicroTube was designed to meet the critical requirements of modern proteomic analysis by mass spectrometry. It is made from fluoropolymer fluorinated ethylene propylene (FEP). This polymer was selected because of its unique features. FEP is highly inert and retains its integrity within an extremely wide temperature range (-200°C to +100°C). The PCT MicroTube's outstanding chemical resistance, non-wetting surface, and negligible protein and nucleic acid binding, help to ensure nearly complete sample recovery - features that are essential for high pressure-enhanced enzymatic proteolysis.



PCT MicroCap

PCT MicroCaps were also designed to meet the critical requirements of modern proteomic analysis by mass spectrometry. They are made from polytetrafluoroethylene (PTFE), a polymer selected for features that are ideal for high pressure applications. The PCT MicroCap is available in three versions for 50, 100, or 150 sample volumes. By selecting a particular MicroCap design, samples may be digested in reaction volumes of approximately 50, 100 and 150 μ L. In addition, all PCT MicroCaps have the unique feature of being usable as gel spot pickers to excise small protein spots from a gel. The PCT MicroCap holds the gel protein spot safely within the cap, while it is transferred to the MicroTube, ready for processing by PCT.



Suggested PCT Workflow

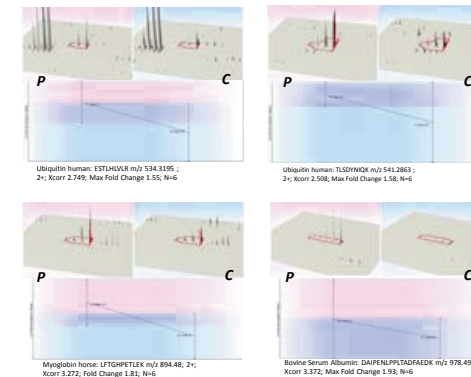
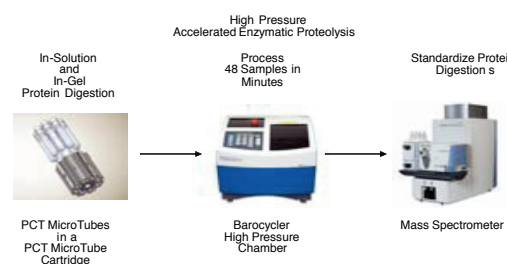


Figure 1. Quantitative profiling of peptide abundance for in-solution digested model proteins. Selected examples illustrate superior digestion efficiency and peptide recovery using PCT.

Data provided by Dr. Alexander Ivanov, Harvard School of Public Health. Special thanks to Dr. Mark Bennett, Nonlinear Dynamics for providing access to the latest version Progenesis LC-MS data analysis software.

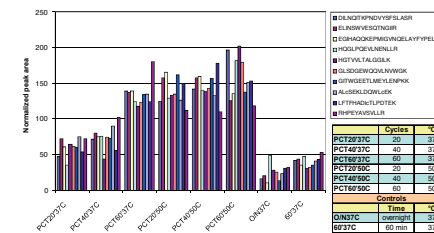


Figure 2. PCT significantly increases digestion efficiency and peptide recovery following in-solution digestion. Digestion was performed using an array of conditions, as indicated. LC-MS was performed on C-18, 10 cm x 150 μ m column using a 30 minute 5-60% gradient (A: 0.1% formic acid; B: acetonitrile, 0.1% formic acid). Peptides were detected on an LTQ-Orbitrap XL mass spectrometer with ETD ionization source; Alternating ETD/CID spectra were collected and the data were analyzed using Proteome discoverer software with 1% FDR data filtering to determine sequence coverage and the total number of peptides. Peak areas for selected peptides were measured using LCQuan package for each LC/MS run (9 runs per digest condition). Values were normalized to the average area obtained for each peptide (n=72). Median values from each set of 9 LC/MS runs for each peptide and each condition are presented.

Data provided by Dr. Roger Biringer, ThermoFischer Scientific

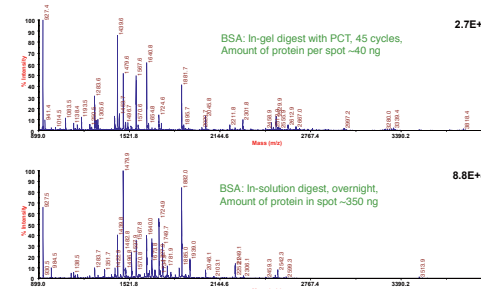


Figure 3. PCT-assisted digestion has been shown to increase efficiency and peptide recovery from gel-separated proteins. New consumable containers offer features which facilitate gel spot picking using a tube closure equipped with a gel cutter tool.

Data provided by Dr. Michail Alterman, CBER, FDA.

Features and Benefits of PCT-enhanced Enzymatic Proteolysis

- Standardize Protein Digestion for Mass Spectrometry
- Substantially Increase Protein Digestion Efficiency
- Protein Digestion in Minutes
- Significantly Accelerate In-Solution and In-Gel Protein Digestion
- Promote Accurate & Efficient Peptide Cleavage
- Avoid Undesired Protein Modifications
- Process Up to 48 Samples at a Time
- Versatile Range of Samples Sizes (50-150 μ L)
- Compatible with a Variety of Proteolytic Enzymes
- Inert, Non-Wetting, Single-Use PCT MicroTube Processing Containers
- Unique PCT MicroCap Design for Protein Spot Transfer from 2D Gels

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