PBI Pressure BioSciences Inc.

PCT NEWS

OTCQB: PBIO

Discovery Starts With Sample Preparation™

Volume 8, Issue 7 July 2014

New Products from PBI for Enhanced Proteomic Research

Extract Protein from Minute Tissue Samples (< 3.0 mg)

Accelerated Protein Digestion in Multiwell Format

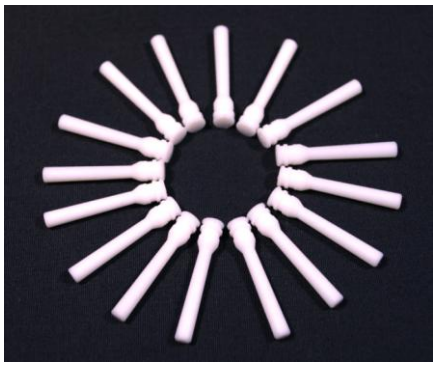
PCT µPestle System

For Use with PCT MicroTubes, PBI Barocyclers, and PCT MicroTube Adapter Kit Workstation

PCT µPestles

PCT µPestles, in conjunction with PCT MicroTubes, are designed to enhance the extraction of protein, DNA, RNA and small molecules from minute amounts (0.5 – 3.0 mg) of solid tissue in extraction reagent volumes as low as 20-30 µL. PCT MicroTubes and PCT µPestles use Pressure Cycling Technology (PCT) to effectively disrupt soft tissues and lyse their cells. Under very high hydrostatic pressure, air is fully dissolved into water - and many materials, like FEP plastics, contract significantly. As a result of these effects, tissue samples placed between the MicroTube end and the µPestle tip is crushed on every pressure cycle. This mechanical action, combined with the extraction ability of the buffer under high pressure, result in effective homogenization and extraction.

PCT µPestles and PCT MicroTubes, together with a PBI Barocycler, comprise the **PCT µPestle System,** which provides a faster, safer, and more efficient means of extraction from extremely small amounts of solid samples such as soft animal tissues or biopsies. The PCT µPestle System can be used in any PBI Barocycler.



Patent Pending

Click on the Title for a Complete Specification Sheet

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The Barozyme™ HT48

The Barozyme HT48, the newest member of PBI's Pressure Cycling Technology (PCT) Barocycler family of products, is a high throughput bench-top instrument designed for accelerated enzymatic digestion of proteins at high pressure. The Barozyme HT48 uses an air-pressure-to-liquid-pressure intensifier system, with a pressure amplification ratio of 160:1, to reach an output pressure of 20,000 psi (~1.4 kbar or ~140 MPa). The Barozyme HT48 is capable of processing up to 48 samples at a time in six single-use BaroFlex 8-well Strips in the Barozyme Sample Carrier. Typical trypsin digestion times can often be reduced from hours to minutes.

Click on the Title for a Complete Specification Sheet

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UPCOMING EVENTS

Scientific Meeting: PBI to showcase the Company's PCT-based systems and new products for sample preparation as well as Constant Systems' cell disruption equipment at the 6th Annual BIOPROCESSING Summit, August 18 - 22, 2014 at the Renaissance Waterfront Hotel in Boston, MA

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PCT µPestle System: Continued from Page 1, Column 1

PCT µPestles are made from polytetrafluoroethylene (PTFE), a synthetic fluoropolymer of tetrafluoroethylene, also known as Teflon (by DuPont Co). PTFE is practically inert. The only chemicals known to affect it are certain alkali metals and most highly reactive fluorinating agents.

PCT µPestles are available in bulk bags of 96, or in racks of 96 with or without MicroTubes. MicroTubes may be purchased separately in similar formats.

Features and Benefits of the PCT µPestle System

- Process 0.5 – 3.0 mg of Soft Solid Tissue in 20-30 µL of Sample Processing Reagent
- Compatible with a Wide Variety of Reagents
- Compatible with a Variety of Enzymes, including Trypsin, PNGase F, Chymotrypsin and Many Others
- Inert, Non-Wetting, Single-Use MicroPestles and MicroTubes
- Available in Several Convenient Easy-to-Use Formats
- PCT MicroTubes Have a Writable Surface

Click on the Title for a Complete Specification Sheet

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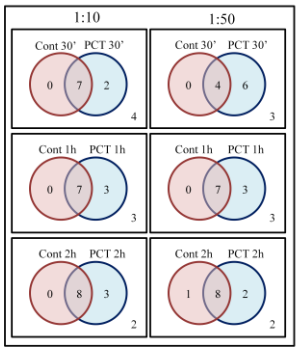
Excerpted from Poster Presented at ASMS 2014

New Products from Pressure BioSciences for Enhanced Proteomic Research

Pressure BioSciences, Inc. 14 Norfolk Ave., South Easton, MA 02375

Digestion Under Pressure: More Peptides and Better Protein Identification

Figure 5. Rapid Digestion of a Model Trypsin-resistant Protein. Pressure-enhanced Digestion vs Control.



Ubiquitin was digested using two E/S ratios (1:10 and 1:50) with pressure (PCT) or without (Cont) in 50mM AMBIC with 10% n-propanol (in triplicate). Pressure cycling was performed at 20,000 psi (~1.4 Kbar or ~140 MPa)

Number of identified peptides is shown. Numeral at the bottom right indicates number of predicted peptides not identified in either digest.

Pressure-enhanced digestion resulted in significantly more peptides in a shorter amount of time.

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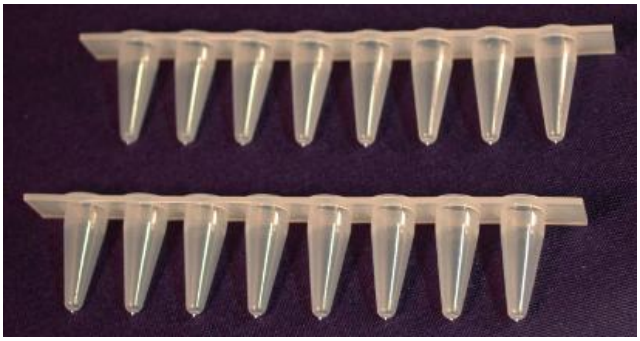
The Barozyme™ HT48: Continued from Page 1, Column 2

BaroFlex 8-well Strips

BaroFlex 8-well strips are used in the Barozyme HT48 (See Specification Sheet) for pressure-enhanced enzymatic digestion at 20,000 psi (~1.4 kbar or ~140 MPa). Only tubes and caps provided by PBI can be used in the Barozyme. Use of non-approved tubes will result in tube breakage, loss of sample under pressure, and harm to the Barozyme HT48 instrument.



Features and Benefits

- Ideal for Accelerated Enzymatic Digestion of Proteins
- Uses Patented Pressure Cycling Technology (PCT)
- Operates at Optimum Pressure of 20,000 psi (~1.4 kbar or ~140 MPa)
- Choice of Run Times for Customizing Digestion Conditions
- Adjustable Chamber Temperature for Added Flexibility
- Uses Convenient "PCR-style" BaroFlex 8-well Strips (Cat # BF8WS-0006.)
- Process Up To 48 Samples at a Time
- Digestion Times Can Often Be Reduced from Hours to Minutes.
- Bench-top High Pressure System with Convenient User Interface
- Laptop with All Necessary Software Provided USB-powered Computer Interface
- Standard 9mm Tube Spacing is Compatible with Standard Multichannel Pipettes, Robotic Liquid Handlers and 96-well Plate Centrifuge Rotors

Click on the Title for a Complete Specification Sheet

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Click on this Link
info@pressurebiosciences.com
to Request a Demonstration of
The PCT µPestle System or
The Barozyme HT48 in Your Laboratory

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Figure 8. Tissue Samples Before and After µPestle Processing with PCT

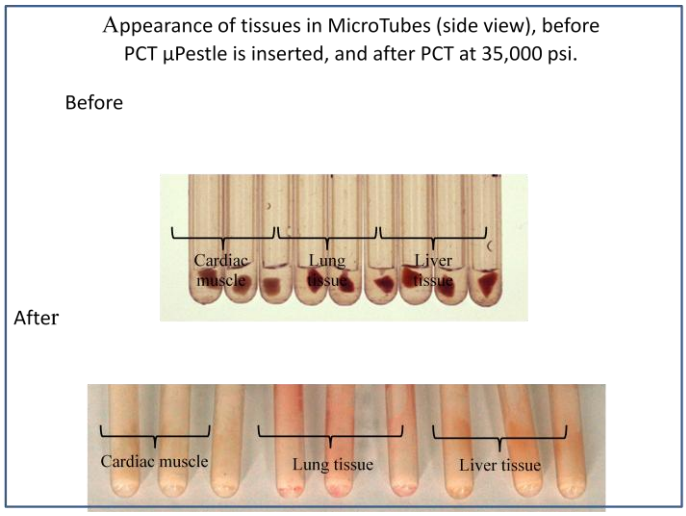

Figure 9. PCT-SWATH: An Integrated Protocol for Accelerated Proteomics from Solid Tissue Samples Using PCT µPestles AND Pressure-accelerated Digestion.

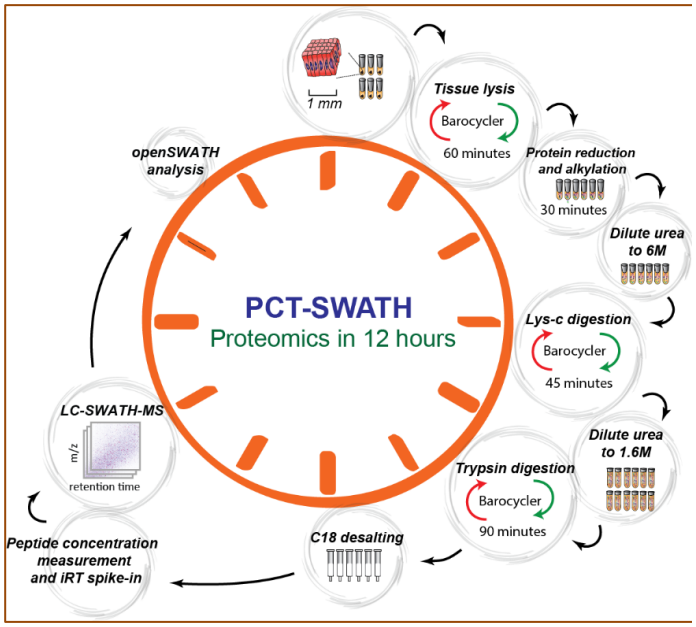

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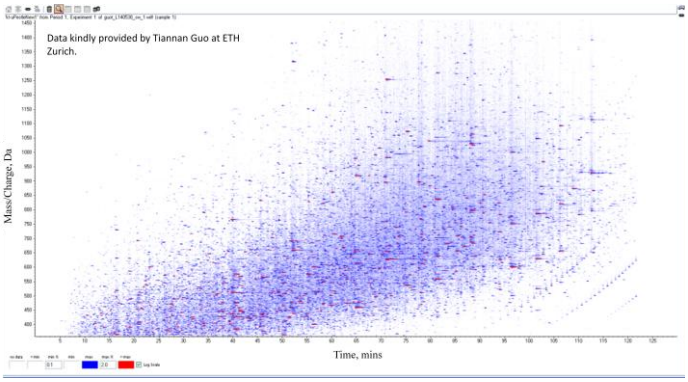
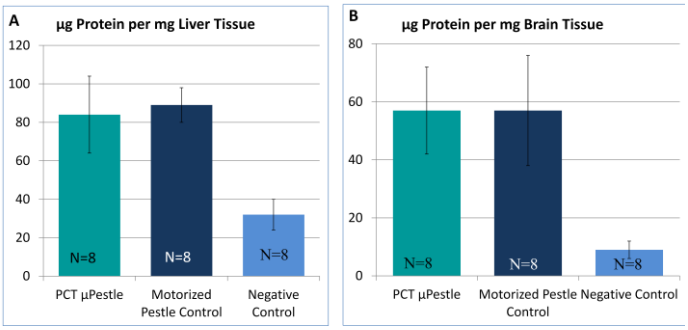
Figure 12. SWATH MS1 Map of Peptides Prepared by µPestle Tissue Homogenization Followed by PCT-SWATH



Human kidney tissue (1-2 mg) was disrupted in lysis buffer using PCT µPestles. The lysate was reduced, alkylated and digested with Lys-C and trypsin in the same MicroTube. The digests were desalted using C18 columns and analyzed by SWATH-MS. Triplicate samples demonstrated high sample-to-sample reproducibility. An example of the dense SWATH MS1 peptide map is shown above (Figure 12).

PCT µPestles: Protein Extraction from Very Small Tissue Samples in MicroTubes Using PCT µPestles and Pressure Cycling



Samples (0.5-2 mg tissue) were disrupted in an NEP 2320 Barocycler by PCT at 35,000 psi (240 MPa/2.4 kbar) for 60 cycles. Negative controls were placed into extraction reagent in MicroTubes, but were not disrupted. Protein concentration in the lysates was measured by Bradford assay (Figure 11). For control extractions, tissue pieces were disrupted with a hand-held motorized Kontes Pellet Pestle Grinder using the same tissue mass and extraction reagent volume as for MicroTubes. This homogenizer provides very effective tissue disruption in a small volume; however, samples must be processed one-at-a-time and the processing is carried out in an open container.

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