# New Products from Pressure BioSciences for Enhanced Proteomic Research

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

Pressure BioSciences Inc. (PBI) focuses on development of products for biological sample preparation which utilize alternating ultra-high hydrostatic pressure – Pressure Cycling Technology (PCT). Pressure is a fundamental thermodynamic parameter which affect a wide range of molecular interactions. PBI's bench-top pressure cycling equipment functions by rapidly and repeatedly raising and lowering pressure in the reaction vessel from atmospheric to levels of up to 100,000 psi (690 Mpa). Pressure cycling equipment and auxiliary tools such as the µPestles described here, have been shown to facilitate more quantitative extraction, more efficient digestion of hydrophobic membrane proteins, and rapid enzymatic removal of N-linked glycans. Here we present an overview of two of PBI's newest products; The Barozyme HT48 for accelerated enzymatic digestion and PCT µPestles for hands-off homogenization of small tissue samples.

## *Barozyme HT48*

The new **Barozyme HT48** from Pressure BioSciences is a high throughput bench-top Pressure Cycling Technology (PCT) instrument designed for accelerated enzymatic digestion of proteins for mass spectrometry applications (Figure 1). The HT48 brings pressure-enhanced digestion to the industry standard microwell plate format for easy automation and interfacing with liquid handling robots. The unit can process up to 48 samples at a time in convenient 8-well strips. Samples are exposed to cycles of high pressure at 20,000 psi (~1.4 Kbar or ~140 MPa) to accelerate digestion and improve detection of difficult to digest proteins. PCT can be performed for 5, 20, 60, 120 or 240 cycles at temperatures up to 60˚C.

Figure 1. Barozyme HT48 with Computer Interface.

## *Barozyme BaroFlex 8-Well Strips and Holder Cassettes*

Specially formulated high density polyethylene (HDPE) BaroFlex 8-well Strips are designed to withstand high pressure when sealed with silicone BaroFlex Cap Strips (not shown) or BaroFlex Mats (Figure 2). Sample wells are easy to cap and un-cap, and can be filled using standard multichannel pipets. The single-use cap strips and mats are designed to seal the tubes tightly and prevent fluid exchange between the sample and the Barozyme chamber fluid during pressure cycling. Storage cap strips are also available that can be used to seal the tubes for storage.

When placed into the base plate (Figure 3), BaroFlex Strips can be centrifuged or processed using robotic liquid handlers. The stainless steel Barozyme sample cassettes hold up to 48 samples in six BaroFlex Strips (Figure 4). The fully assembled cassette fits into the sample chamber for processing at 20,000 psi (~1.4 Kbar or ~140 MPa).

Figure 2. BaroFlex 8-well Strips and BaroFlex 24-Cap Mat.

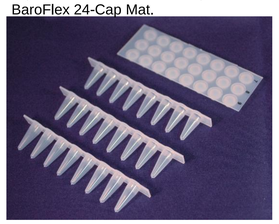
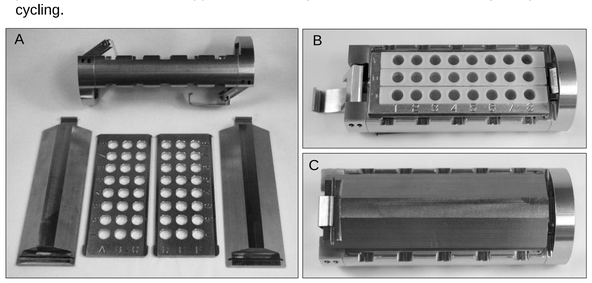


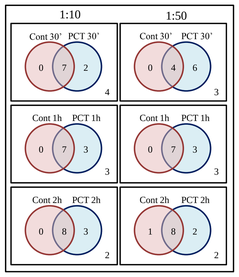
Figure 3. Base Plate with Holder and Three BaroFlex Strips.

Figure 4. BaroFlex Strip Holder Cassette.
**A**. Disassembled cassette with 2 lids and 2 strip holder plates. **B**. Partly assembled cassette showing internal arrangement with three BaroFlex Strips in strip holder plate placed into cassette. **C**. Appearance of fully assembled cassette ready for pressure cycling.




## *Digestion Under Pressure: More Peptides and Better Protein Identification.*

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

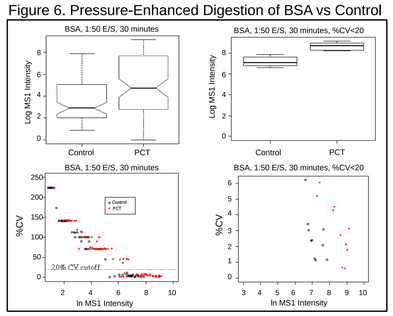



Ubiquitin was digested using two E/S ratios (1:10 and 1:50) with pressure (PCT) or without (Cont) in 50mM AMBIC with 10% n-propanol (in triplicate). Pressure cycling was performed at 20,000 psi (~1.4 Kbar or ~140 MPa)

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.

Figure 6. Pressure-Enhanced Digestion of BSA vs Control




BSA was digested with trypsin (1:50 E/S) for 30 minutes with pressure at 20,000 psi (~1.4 Kbar or ~140 MPa) (PCT) or under standard conditions (Cont). Pressure-enhanced digestion resulted in significantly greater intensities for most identified peptides. These peptides covered 30.7% and 22.8% of BSA sequence, respectively. A greater number of peptides suitable for reliable quantitative analysis (C.V.≤ 20%) was obtained using the pressure-enhanced digestion workflow (red) compared to control (black), thus offering greater throughput for targeted proteomics workflows.

## *Barozyme HT48 Features, Benefits and Technical Specifications*

- Run up to **48 samples** at a time.
- Sample volume of 140 µl per tube.
- Easy temperature control.
- Preset pressure parameters for ease-of-use and consistent results.
- User settable run time from 5 minutes to 4 hours.
- Digestion times can be reduced from hours to minutes.
- Improved digestion of hydrophobic and difficult-to-digest proteins.
- Easy filling of BaroFlex 8-well Strips using standard pipet tips and single or multichannel pipets.
- Industry standard 9mm spacing allows for easy interfacing with robotic liquid handlers.
- Single-use BaroFlex Strips and BaroFlex Caps do not require cleaning between samples.
- BaroFlex Strips can be centrifuged in standard 96-well plate centrifuge rotors.

## *PCT µPestles: Design and Applications*

PBI's new, patent-pending PCT µPestles, in conjunction with PCT MicroTubes (Figure 7), are designed for hands-off homogenization of 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 disrupt and homogenize soft tissues (Figure 8). Under very high hydrostatic pressure the tissue sample is trapped between the bottom of the MicroTube and the PCT µPestle tip and is crushed on each pressure cycle. This mechanical action, combined with the effect of extraction buffer under high pressure, result in effective hands-off homogenization and extraction.

Figure 7. PCT µPestles (A), MicroTubes (B) and Cartridges (C).

Figure 8. Tissue samples before and after µPestle processing with PCT.

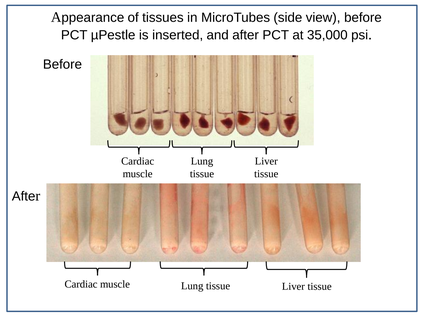
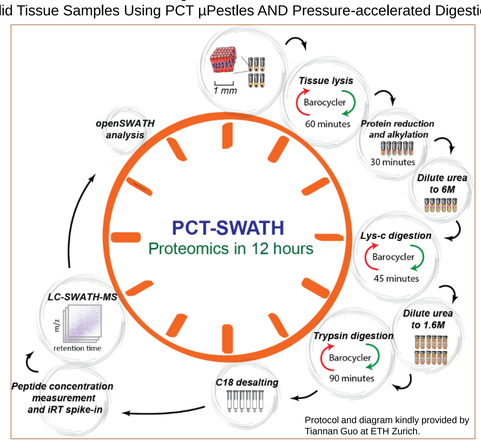



Figure 9. PCT-SWATH: An Integrated Protocol for Accelerated Proteomics from Solid Tissue Samples Using PCT µPestles AND Pressure-accelerated Digestion.

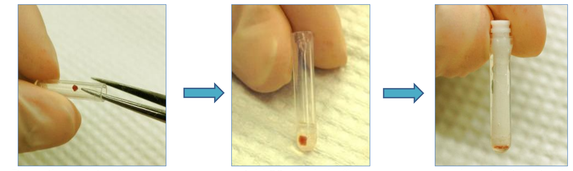



Protocol and diagram kindly provided by Tiannan Guo at ETH Zurich.

**Summary**
The PCT µPestle is an effective tool for rapid and convenient disruption and extraction of small solid tissue samples. Single-tube protocols, such as PCT-SWATH allow for significantly reduced hands-on time, good reproducibility, and increased throughput, when compared to manual homogenization and standard overnight trypsin digestion.

## *PCT µPestles: Simple and Convenient Workflow.*

Figure 10. µPestle Workflow



Place tissue into MicroTube

Add 30µl lysis buffer of choice

Insert PCT µPestle

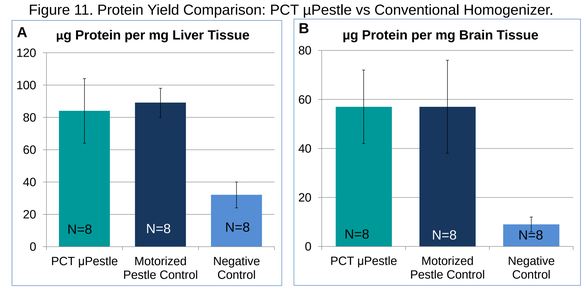
Recover lysate from MicroTubes by pipetting. Alternatively, MicroTubes can be inverted into small centrifuge tubes and centrifuged briefly to easily collect the homogenate without pipetting.

Pressure-enhanced trypsin digestion of the whole lysate can be performed directly in the same MicroTubes, as for PCT-SWATH.

Process in Barocycler

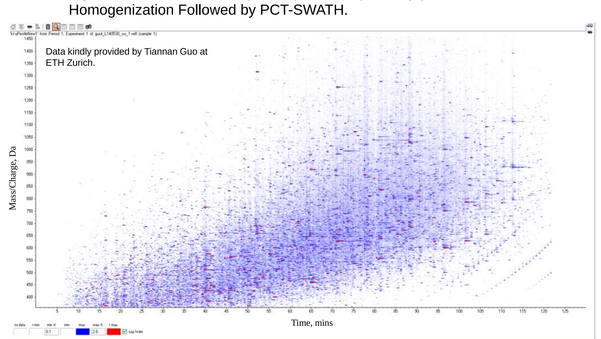
## *PCT µPestles: Protein Extraction from Very Small Tissue Samples in MicroTubes Using PCT µPestles and Pressure Cycling*

Figure 11. Protein Yield Comparison: PCT µPestle vs Conventional Homogenizer.




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.

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 a 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).

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