

# New Products from Pressure BioSciences for Enhanced Proteomic Sample Preparation

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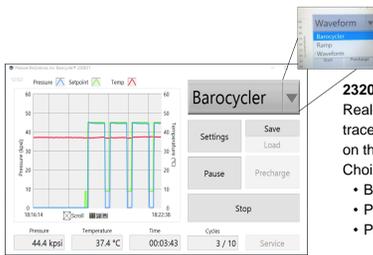
## Barocyler 2320EXT

The new Barocyler 2320EXT from Pressure BioSciences is a next generation, benchtop, Pressure Cycling Technology (PCT) instrument, designed for PCT-HD and other workflows that call for homogenization/extraction from tissue samples, and/or accelerated enzymatic digestion of proteins for mass spectrometry and other applications. The unit can process up to 16 samples at a time using PCT microTubes. Samples are exposed to cycles of pressure up to 45,000psi to improve extraction and accelerate digestion for better, faster, and more consistent detection of difficult-to-digest proteins.

## 2320EXT User Interface and Software Control

### Features of the 2320EXT Hardware and Software

- Bench top high pressure system
- Touch screen user interface
- Real-time pressure and temperature read-out
- Automatic logging of all run parameters
- Flexible run times and pressure cycling parameters for customizing extraction and digestion conditions
- Choice of temperature control (integrated electric heater or external circulating waterbath)
- Up to 16 samples per run in PCT MicroTubes
- Pressures up to 45,000 psi (310 MPa)



### 2320EXT User Interface

Real-time readouts of pressure (green/blue trace) and temperature (red trace) displayed on the software Main Screen.

Choice of three pressure cycling modes:

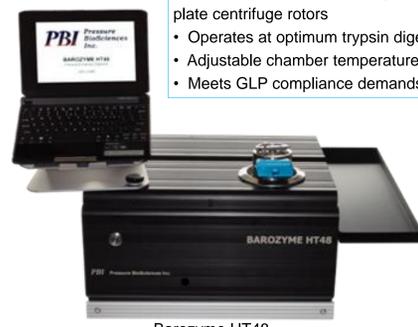
- Barocyler
- Pressure ramping
- Pressure waveform

## Benefits of the Barocyler 2320EXT

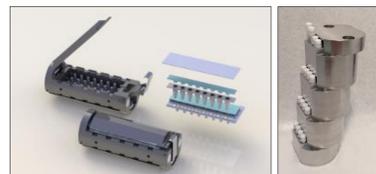
- Run up to **16 samples** at a time in PCT MicroTubes.
- Pressure chamber temperature up to 95°C.
- User settable run times and flexible pressure cycling parameters
- Digestion times can often be reduced from 12-18 hours to 1 hour or less.
- Improved digestion of hydrophobic and difficult-to-digest proteins.
- Single-use MicroTubes, MicroCaps and MicroPestles do not require cleaning between samples.
- Pressures up to 45,000 psi (310 MPa) for optimized digestion with a variety of enzymes, such as:
  - Trypsin at 20,000psi
  - Lys-C at 45,000psi
  - Chymotrypsin at 45,000psi

## The Barozyme™ HT48, another member of the Barocyler Family of Products

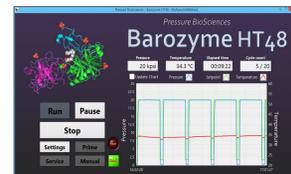
- Bench-top high pressure system with USB-powered computer interface
- Uses the patented Pressure Cycling Technology (PCT) Platform.
- Ideal for high throughput accelerated enzymatic digestion of proteins for mass spec and other applications
- Reduces trypsin digestion time to 90 minutes or less, for most applications
- Two throughput options
  - Up to 48 samples per run using 'PCR-style' BaroFlex 8-well strips
  - Up to 96 samples per run using PCT Microtubes
- Universal 9mm tube spacing with the BaroFlex strips is compatible with standard multichannel pipettes, robotic liquid handlers, and 96-well plate centrifuge rotors
- Operates at optimum trypsin digestion pressure of 20,000psi
- Adjustable chamber temperature and choice of run times for customized digestions
- Meets GLP compliance demands of biopharmaceutical, clinical proteomics, and other regulated laboratories



Barozyme HT48



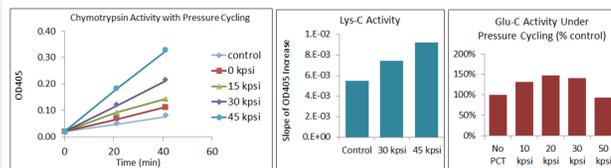
Run up to 48 samples at a time in 8-well BaroFlex strips, or up to 96 at a time in PCT MicroTubes



Easy-to-use user interface with real-time pressure and temperature graph

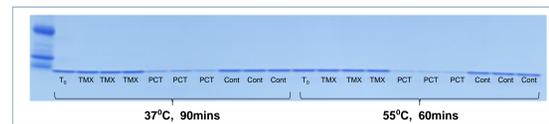
Pressure BioSciences Inc. (PBI) focuses on development of products for biological sample preparation that utilize alternating ultra-high hydrostatic pressure – Pressure Cycling Technology (PCT). Pressure is a fundamental thermodynamic parameter that affects a wide range of molecular interactions. PBI's benchtop pressure cycling equipment functions by rapidly and repeatedly raising and lowering hydrostatic pressure in the reaction vessel from atmospheric (14.7psi) to levels of up to 90,000 psi (690 MPa). Pressure cycling equipment and auxiliary tools such as the PCT MicroPestles, have been shown to facilitate more quantitative extraction, more efficient digestion of hydrophobic membrane proteins, and more rapid enzymatic removal of N-linked glycans. Here we present an overview of some of PBI's newest products: The **Barocyler 2320EXT** and how it can be used with the **PCT-HD** workflow for improved proteomic sample preparation of small samples such as tissue biopsies.

## Digestion Under Pressure: Faster Digestion, More Peptides and Better Protein Identification

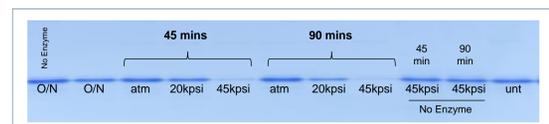


**PCT accelerates a variety of proteolytic enzymes, including chymotrypsin, Lys-C and Glu-C. Different enzymes have different optimal pressure levels for accelerated activity.**

Pressure effects on enzymatic activity of chymotrypsin, Lys-C, and Glu-C were measured spectrophotometrically using appropriate chromogenic substrates to eliminate any influence of pressure on protein substrate conformation. All three enzymes demonstrate increased activity at elevated pressures.



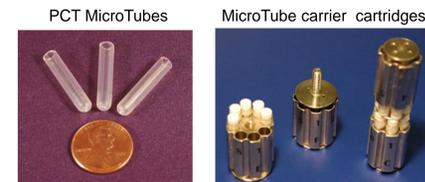
**Trypsin Digestion of Native Ubiquitin is Accelerated by PCT at 20kpsi.** Digest reactions were incubated at 37°C for 90mins or at 55°C for 60mins. Pressure cycling (PCT) was performed at 20,000 psi. Thermomixer (tmx) vs. PCT vs. control. Disappearance of the parent protein band is indicative of enzymatic digestion.



**Lys-C Digestion of Native Myoglobin is Accelerated by PCT at 45kpsi.** Digest reactions were incubated for 45 or 90mins at atmospheric pressure (atm) or with pressure cycling at 20,000 or 45,000 psi. Control reactions were incubated overnight (O/N) with or without enzyme. An untreated control (unt) is also shown. Disappearance of the parent protein band is indicative of enzymatic digestion.

## PCT MicroTubes and MicroCaps: Convenience and Flexibility

- Versatile range of sample volumes (50, 100, 150 µl) for liquid or suspension samples
- Unique MicroCap design helps to displace air from sample tubes
- Compatible with a variety of extraction reagents
- Inert, non-wetting, single-use PCT MicroTubes are made of FEP

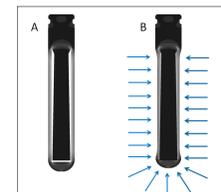


## PCT MicroPestles for Hands-off Tissue Homogenization and Protein Extraction

- Homogenize and extract protein from soft solid tissue samples.
- Small sample sizes, such as tissue biopsies (up to 3-5mg).
- Small volume of extraction reagent reduces sample dilution.
- Inert, non-wetting, single-use MicroPestles and MicroTubes are compatible with a wide variety of extraction reagents.
- Hands-off homogenization/extraction of up to 16 samples per run.
- Homogenization, reduction/alkylation, and digestion can be carried out in a single MicroTube, reducing sample loss and dilution.



PCT MicroPestles



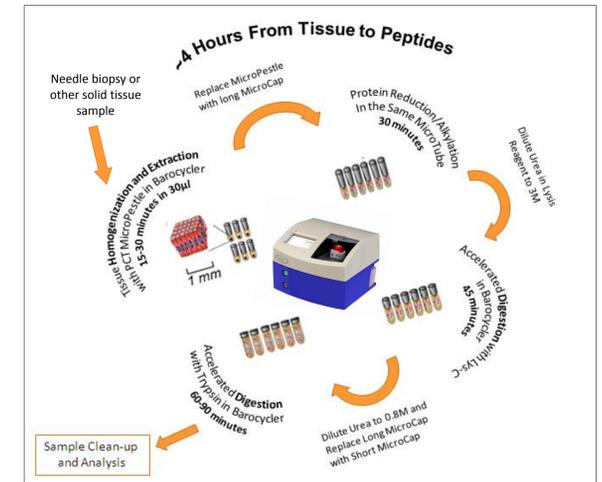
### MicroPestle tissue homogenization mechanism:

MicroPestle and tube at ambient pressure (A) and under high hydrostatic pressure (B). At each pressure cycle the tube is compressed, causing it to narrow and shorten. This mechanical action, combined with the effect of extraction buffer under high pressure, results in effective hands-off homogenization and extraction.

## Summary

The PCT-HD System combines the hands-off multi-sample throughput of the PCT MicroPestle, with pressure-accelerated digestion, to significantly reduce processing time for MS workflows. Protein extraction with the PCT MicroPestle from biopsy-size tissues is rapid and convenient, providing excellent reproducibility and increased throughput. Pressure-accelerated digestion can be performed in the same MicroTube sample container to minimize sample transfers, improve yields, and save time. In addition, samples extracted and digested at high pressure may yield more peptides from difficult-to-extract and/or difficult-to-digest targets which may be underrepresented in traditional sample preparations.

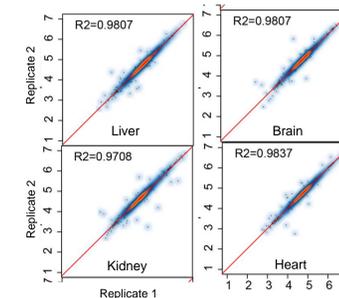
## PCT-HD: A PCT-Enhanced Tissue Homogenization, Protein Extraction, and Digestion System for Mass Spectrometry Sample Preparation



### Single-tube PCT-HD Workflow:

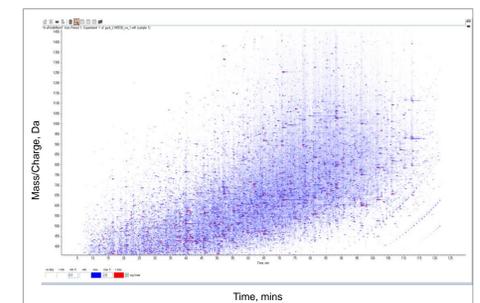
- 30 minute tissue homogenization at 35,000-45,000psi
- Reduction/alkylation (optional) at ambient pressure
- 3.45 minute Lys-C digestion (optional) at 45,000psi
- 60-90 minute trypsin digestion at 20,000psi

## PCT-HD Sample Preparation for SCIEX SWATH-MS



### Excellent sample-to-sample reproducibility with PCT-HD.

Proteins were extracted using PCT MicroPestles from liver, kidney, brain and heart tissues. Extracts were digested with lys-C followed by trypsin using the PCT-HD workflow described above, and subjected to SWATH-MS. Duplicate biological replicates were compared and demonstrate excellent sample-to-sample reproducibility (log intensity shown on X and Y axes).



### SWATH MS1 Map of Peptides Prepared by PCT-HD and Analyzed by SWATH-MS

Human kidney tissue (1-2 mg) was disrupted in lysis buffer using PCT MicroPestles. 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.

MS data kindly provided by Dr. Tiannan Guo at ETH Zurich, Switzerland and Dr. Shiyong Shao at Huazhong University of Science and Technology, Wuhan, PRC.