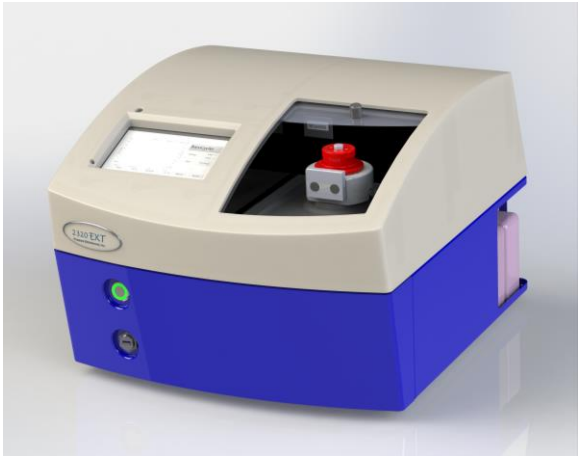
Barocycler® 2320EXT

For the PCT EXTREME Sample Preparation System

Pressure Cycling Technology (PCT) is a unique, patented technology platform based on repeated cycles of hydrostatic pressure between ambient (14.7 psi) and ultra-high levels (up to 45,000 psi). These rapid changes in pressure are used to control biomolecular interactions for applications such as accelerated proteolytic digestion and improved extraction of cellular components, including proteins and lipids. The PCT platform allows for a high degree of safety, speed, reproducibility, and convenience in modern sample preparation protocols.

PCT-HD is a proven PCT-based workflow for processing small tissue samples. PCT-HD combines efficient, hands-off tissue homogenization and protein extraction with pressure-enhanced digestion, for the rapid, efficient and reproducible generation of peptides for MS analysis. This unique workflow combines two of PBI's innovative sample preparation tools: the new Barocycler 2320EXT (EXTREME) and our patent-pending PCT MicroPestles. PCT-HD with the new Barocycler 2320EXT can provide unprecedented speed and reproducibility for biomarker discovery, yielding peptides ready for clean-up and MS within 2-4 hours from the start of tissue processing.

The new Barocycler 2320EXT instrument replaces the NEP2320-ENH Barocycler in the PCT-HD workflow. The 2320EXT incorporates superior pressure and temperature control for the next generation of high pressure sample preparation. Like the NEP2320-ENH, the 2320EXT is a compact, bench top instrument that can process up to 16 samples simultaneously using PCT MicroTubes. Improvements that come standard with the 2320EXT include: increased pressure and temperature ranges to support unparalleled protein extraction from fresh, frozen, and FFPE-treated tissues; data input and output options to accommodate validation and quality control; a choice of two different methods for temperature control (an external circulating waterbath or a built-in electric heater); computer-operated control with touch screen programming and automatic data logging; and the ability to control multiple pressure cycling parameters, such as the rate of pressure increase/decrease, both the high and low pressure levels, and the shape of the pressure profile (e.g., sine wave, square wave, and others), ideal for basic research.
Patent Pending



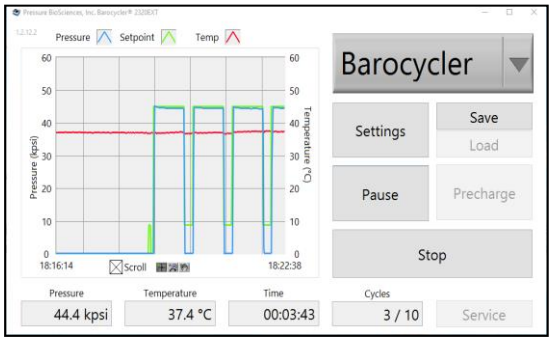
The Barocycler 2320EXT

- Pressure Range: ambient - 45,000psi (310 MPa)
- Max Temperature: 95˚C
- Min Temperature: 4˚C with circulating waterbath, ambient with electric heating
- Insulated closure for use during high temperature runs
- Process up to 16 samples simultaneously
- Easily accessible USB port on front panel
- Choice of temperature control - heating/cooling or heating only
- Requires 110psi input air pressure
- Computer-operated control with Touch Screen Interface
- Pressure medium: Distilled Water

Touch Screen Interface

Pressure Profiles are programmed via an onboard touch screen, which can be connected to a network. The instrument is also equipped with a USB port that can be used to connect a mouse or keyboard to the 2320EXT.

- Various Pressure Mode Settings: Barocycler, Ramp, Waveform
- Cycled or Static Pressure
- Network Enabled
- Automatic Logging of All Run Parameters
- Data Output
- Save and Record Programs
- Real-time pressure display graph shows pressure and temperature throughout the run
- Touch Screen Temperature Control (with electric heating option only).



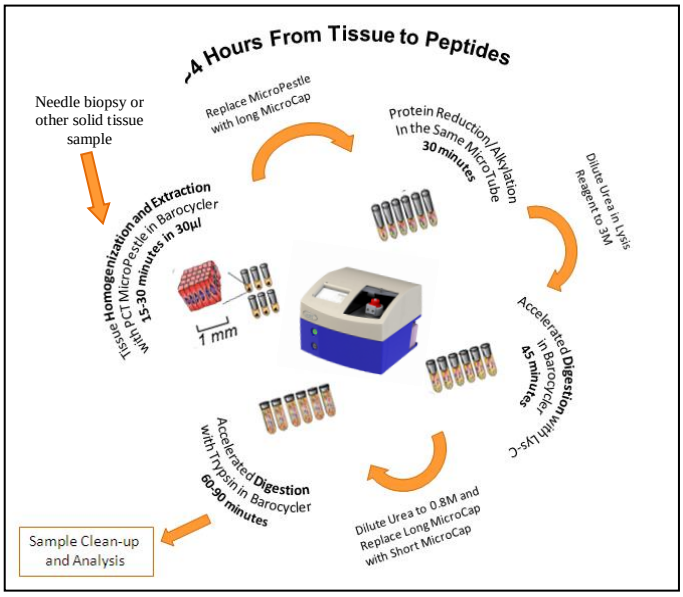
MicroTubes and MicroPestles

MicroTube Adapter Cartridges are included with the Barocycler 2320EXT. These are essential for use with PCT MicroTubes and PCT uPestles[9].

PCT µPestles, in conjunction with PCT MicroTubes, are designed to enhance homogenization and extraction when working with minute amounts of solid tissue.

- Process up to 3mg of solid tissue in 30uL of extraction reagent
- Process as few as 5 x 10^4 cells
- MicroTubes are constructed of non-wetting, low binding FEP plastic
- MicroPestles are made from inert PTFE (Teflon)

PCT-HD Work Flow with the Barocycler 2320EXT



PCT-HD was developed by PBI scientists and engineers in collaboration with Professor Ruedi Aebersold and Dr. Tiannan Guo of the Institute of Molecular Systems Biology, ETH Zurich, and the University of Zurich, Zurich, Switzerland [1, 2, 3, 4, 5, 6, 7, and 8].

References (Hyperlinked)

1. Reproducible Tissue Homogenization and Protein Extraction for Quantitative Proteomics using MicroPestle-assisted Pressure Cycling Technology. *Shiying Shao, et al.*
2. Rapid mass spectrometric conversion of tissue biopsy samples into permanent quantitative digital proteome maps *Tiannan Guo, et al.*
3. Quantifying cancer related proteins via targeting MS assay resources *Ruedi Aebersold, Ph.D.*
4. Proteomic investigation of intra-tumor heterogeneity *Tiannan Guo, MD, Ph.D.*
5. Proteotypic features of NCI-60 cancer cells obtained by SWATH-MS predicts phenotypes *Tiannan Guo, et al.*
6. Analysis of Tissue Biopsies by PCT-SWATH *Tiannan Guo, MD, Ph.D.*
7. *Hands Free Sample Homogenization and Protein Extraction from Small Tissue Biopsy Using Pressure Cycling Technology and MicroPestle Shiying Shao et al.*
8. *More Efficient Tissue Lysis and Protein Digestion with Lower Concentration of Denaturant Using Pressure Cycling Technology Wen Yan et al.*
9. PCT µPestle System Specification Sheet

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