Pressure BioSciences Inc.

PCT NEWS

OTCQB: PBIO
Discovery Starts With Sample Preparation™

Volume 9, Issue 9 September 2015

Pressure BioSciences to Showcase PBI's PCT-HD System for PCT-SWATH at the HUPO World Congress 2015

Excerpts from PCT-SWATH Related Publications and Posters

Click on Titles for Full Publications

Mass spectrometry-based proteomic quest for diabetes biomarkers

Rapid mass spectrometric conversion of tissue biopsy samples into permanent quantitative digital proteome maps

Stratification of Histologically Indistinguishable Prostate Cancer Tissue Biopsy Samples Using Quantitative Proteotypes Obtained by PCT-SWATH

Minimal sample requirement for highly multiplexed protein quantification in cell lines and tissues by PCT-SWATH mass spectrometry

Hands Free Sample Homogenization and Protein Extraction from Small Tissue Biopsy Using Pressure Cycling Technology and MicroPestle

PCT-SWATH Using PBI's PCT-HD System

A PCT-enhanced Tissue Homogenization, Protein Extraction, and Accelerated Digestion System for Mass Spectrometry Sample Preparation

The PCT-HD System (Pressure Cycling Technology-High Definition) combines two of PBI's unique products: the new Barocycler NEP2320-Enhanced instrument and the PCT µPestle System for homogenization and accelerated digestion of tissue for biomarker discovery. PCT-HD was developed by PBI scientists and engineers in collaboration with Professor Ruedi Aebersold, Dr. Tiannan Guo, and the Aebersold Lab of ETH Zurich, Switzerland. Drs. Aebersold and Guo combined PCT-HD with AB Sciex's SWATH-Mass Spectrometry and named the resulting method "**PCT-SWATH**". This protocol can yield analytical results within 12 hours from the start of tissue processing. Although Drs. Aebersold and Guo developed protocols for the combination of PCT-HD with SWATH-MS, the PCT-HD System is not limited to any specific mass spectrometer or method of data analysis. Regardless of the analytical system that is used, the PCT-HD System can provide researchers with unprecedented speed and reproducibility for biomarker discovery.

PCT µPestles* and PCT µTubes

PCT µPestles are designed for homogenization and extraction of minute amounts of tissue (up to 3 mg) in as little as 30 µL of lysis buffer. During PCT, tissue trapped between the flexible µTube and the rigid µPestle is repeatedly crushed in each pressure cycle. This mechanical action, combined with an extraction buffer under high pressure, results in extremely effective homogenization and extraction of tissue.

The small size and inert materials of the PCT µTubes and PCT µPestles help to reduce sample loss during processing and ensure nearly complete sample recovery, essential when working with minute or precious samples.

***Patent Pending**

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Excerpted from Biochimica et Biophysica Acta
Volume 1854, Issue 6, June 2015, Pages 519–527

Mass spectrometry-based proteomic quest for diabetes biomarkers

Shiying Shao[a,b], Tiannan Guo[b], and Ruedi Aebersold[b,c]
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ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia, which affects hundreds of millions of individuals worldwide. Early diagnosis and complication prevention of DM are helpful for disease treatment. However, currently available DM diagnostic markers fail to achieve the goals. Identification of new diabetic biomarkers assisted by mass spectrometry (MS)-based proteomics may offer solution for the clinical challenges. Here, we review the current status of biomarker discovery in DM, and describe the pressure cycling technology (PCT)-Sequential Window Acquisition of all Theoretical fragment-ion (SWATH) workflow for sample-processing, biomarker discovery and validation, which may accelerate the current quest for DM biomarkers. This article is part of a Special Issue entitled: Medical Proteomics.

Click on Title for Complete Article

UPCOMING EVENTS

Pressure BioSciences, Inc. (PBI) to showcase its new cutting-edge Barozyme HT48 high throughput PCT-based instrument for enhanced enzymatic digestion of proteins, and its new PCT-HD System for both protein extraction and accelerated digestion from biopsy-sized tissue samples at the HUPO 14th Annual World Congress (HUPO 2015), September 28 – 30, 2015, Vancouver Convention Center, Vancouver, British Columbia, Canada.

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PCT-SWATH Using PBI's PCT-HD System: **Continued from Page 1, Column 1**

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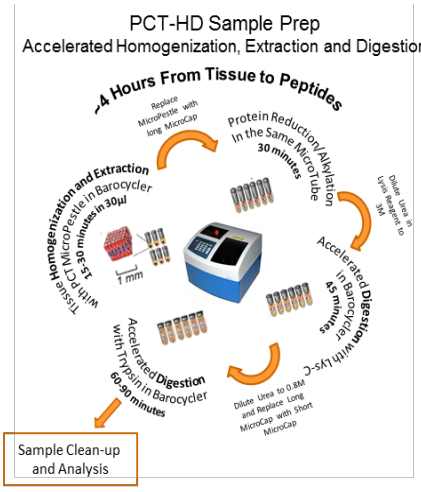
MicroPestles **MicroTubes**

Barocycler NEP2320-Enhanced

To get the full power of PCT-HD for protein extraction and accelerated digestion, PBI developed the Barocycler NEP2320-Enhanced. Although the basics of the PCT-HD method can be performed in any PBI Barocycler, the new Barocycler NEP2320-Enhanced is designed to increase throughput and offers a broader range of pressure and temperature options, compared to the standard NEP2320 unit.



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Excerpted from Nature Medicine 21, 407–413 (2015)
doi:10.1038/nm.3807

Rapid mass spectrometric conversion of tissue biopsy samples into permanent quantitative digital proteome maps

Tiannan Guo[1], Petri Kouvonen[1,2], Ching Chiek Koh[1,3], Ludovic C Gillet[1], Witold E Wolski[1,4], Hannes L Röst[1,5], George Rosenberger[1,5], Ben C Collins[1], Lorenz C Blum[4,6], Silke Gillessen[7], Markus Joerger[7], Wolfram Jochum[8] & Ruedi Aebersold[1,9].

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Abstract

Clinical specimens are each inherently unique, limited and nonrenewable. Small samples such as tissue biopsies are often completely consumed after a limited number of analyses. Here we present a method that enables fast and reproducible conversion of a small amount of tissue (approximating the quantity obtained by a biopsy) into a single, permanent digital file representing the mass spectrometry (MS)-measurable proteome of the sample. The method combines pressure cycling technology (PCT) and sequential window acquisition of all theoretical fragment ion spectra (SWATH)-MS. The resulting proteome maps can be analyzed, re-analyzed, compared and mined *in silico* to detect and quantify specific proteins across multiple samples. We used this method to process and convert 18 biopsy samples from nine patients with renal cell carcinoma into SWATH-MS fragment ion maps. From these proteome maps we detected and quantified more than 2,000 proteins with a high degree of reproducibility across all samples. The measured proteins clearly distinguished tumorous kidney tissues from healthy tissues and differentiated distinct histomorphological kidney cancer subtypes.

Excerpted from Poster ASMS 2015

Stratification of prostate cancer tissue biopsy samples using PCT-SWATH

Tiannan Guo[1]; Li Li[2]; Ulrich Wagner[3]; Qing Zhong[3]; Christine Fritz[3]; Christian Fankhauser[3]; Cedric Poyet[3]; Rebecca Hartmann[3]; Andreas Beyer[2]; Peter Wild[3]; Ruedi Aebersold[1, 4]

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Prostate cancer (PC) patients are conventionally classified into three histological groups, i.e. low-grade, intermediate-grade and high-grade. The intermediate-grade group contains patients of mixed clinical outcomes that cannot be better classified using established diagnostic routines.

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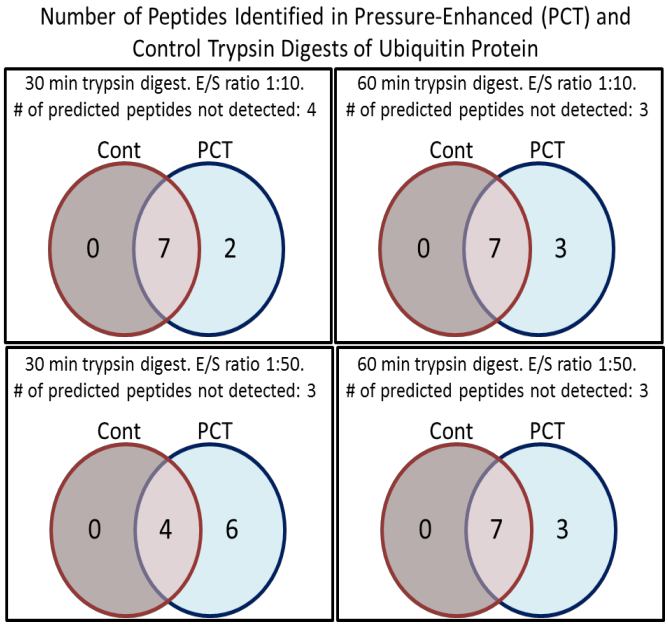
PCT-SWATH Using PBI's PCT-HD System: **Continued from Page 2, Column 1**



Features and Benefits of PCT-HD

- Pressure up to 45 kpsi (310 MPa) and Temperature
- up to 90°C
- Process up to 16 Samples Per Run
- Compatible with a Wide Variety of Extraction and Digestion Reagents
- Accelerated and Improved Digestion of Proteins with a Variety of Enzymes Including Trypsin, Lys-C, PNGase F, Chymotrypsin and Many Others
- Sequential Pressure-Enhanced Digestion Using Lys-C and Trypsin
- Better Digestion of Many "Difficult-to-Digest" Proteins
- Automated Homogenization Reduces Variability
- Reduced Sample Loss and Cross-Contamination

Request More Information

Stratification of prostate cancer tissue biopsy samples using PCT-SWATH: **Continued from Page 2, Column 2**

Here, we hypothesize that the quantitative proteomic analysis of tissue biopsy samples could be used to stratify intermediate-grade PC patients.

To obtain accurate quantitative proteotypes (the acute state of the proteome) of tissue biopsy samples, we used the recently developed combination of PCT (pressure cycling technology) and SWATH-MS (reference 1), which permits relative quantification of thousands of proteins from tissue biopsies with reproducibility and quantitative accuracy comparable to SRM, the gold standard quantitative MS method.

Excerpted from Proteomics, DOI: 10.1002/pmic.201500161,
Article first published online: 10 SEP 2015

Minimal sample requirement for highly multiplexed protein quantification in cell lines and tissues by PCT-SWATH mass spectrometry

Shiying Shao[1,2], Tiannan Guo[2], Chiek Ching Koh[2], Silke Gillessen[3], Markus Joerger[3], Wolfram Jochum[4] and Ruedi Aebersold[2,5,*]

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Introduction

The amount of sample available for clinical and biological proteomic research is often limited and thus significantly restricts clinical and translational research. Recently, we have integrated pressure cycling technology (PCT) assisted sample preparation and SWATH-MS to perform reproducible proteomic quantification of biopsy-level tissue samples. Here, we further evaluated the minimal sample requirement of the PCT-SWATH method using various types of samples, including cultured cells (HeLa, K562, and U251, 500 000 to 50 000 cells) and tissue samples (mouse liver, heart, brain, and human kidney, 3–0.2 mg). The data show that as few as 50 000 human cells and 0.2–0.5 mg of wet mouse and human tissues produced peptide samples sufficient for multiple SWATH-MS analyses at optimal sample load applied to the system. Generally, the reproducibility of the method increased with decreasing tissue sample amounts. The SWATH maps acquired from peptides derived from samples of varying sizes were essentially identical based on the number, type, and quantity of identified peptides. In conclusion, we determined the minimal sample required for optimal PCT-SWATH analyses, and found smaller sample size achieved higher quantitative accuracy.

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Excerpted from Poster USHUPO 2015

Hands Free Sample Homogenization and Protein Extraction from Small Tissue Biopsy Using Pressure Cycling Technology and MicroPestle

Shiying Shao[1,2]; Vera Gross[3]; Wen Yan[1,2]; Tiannan Guo[2]; Alexander Lazarev[3]; Ruedi Aebersold[1,4]

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Abstract

Efficient homogenization of small amounts of samples, such as biopsy-level tissues, and reproducible protein extraction from these samples, remain outstanding technical challenges for protein analyses in clinical research, particularly in the coming era of precision medicine. We have recently presented an integrated method based on pressure cycling technology and pressure-resistant MicroTubes to efficiently perform tissue homogenization and protein extraction with minimal manual operation, assisted by the Barocycler.

In order to further enhance the efficiency of this method, here we present an improved extraction method which utilizes the PCT µPestle, a disposable mechanical tissue homogenizer that is incorporated directly into the MicroTube sample container. The container walls collapse around the insert under repeated pulses of high hydrostatic pressure, resulting in a homogenizer-like effect. Pressure cycling also acts directly on the sample, resulting in more efficient tissue lysis.

We optimized the pressure cycling conditions for µPestle, and further evaluated the performance of the optimized µPestle method using our conventional MicroCap method as a benchmark. Comparison of extraction yields from the two methods using mouse liver, heart, and brain tissues of different amounts (ranging from 0.55 mg to 13 mg) led to the same conclusion, that µPestle extracted significantly more proteins than the MicroCap method.

We further integrated the µPestle method into the PCT-based protein digestion and SWATH-MS analysis workflow [1], and found the optimized pestle method produced 20-40% more peptides for various tissues and identified more biologically informative proteins after SWATH analysis when compared to the MicroCap method. In conclusion, we have developed a hands-free method for efficient and reproducible tissue homogenization and protein extraction, and integrated it into the PCT-SWATH workflow which rapidly converts small amounts of sample into a digital map representing the MS-measurable proteome.

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Pressure BioSciences' PCT-HD System (PCT High Definition)

A Tissue Homogenization, Protein Extraction and Accelerated Digestion System for Biomarker Discovery and Mass Spectrometry Sample Preparation

Barocycler NEP2320-Enhanced
(45 kpsi-Elevated Temperature to 90°C)

MicroPestles and MicroTubes

PCT-HD Specification Sheet

Request Information

Features and Benefits

- Extract Proteins from Minute Tissue Biopsy Samples (0.2 - 3.0 mg of Soft Solid Tissue)
- Unprecedented Speed and Reproducibility with Pressure Cycling Technology (PCT)
- Rapid Digestion of Proteins (Minutes vs. Hours)
- Compatible with a Variety of Enzymes, including Trypsin, Lys-C, PNGase F, Chymotrypsin and Many Others
- Process Up to 16 Samples Simultaneously
- Inert, Single-Use MicroPestles and MicroTubes

PCT-HD was developed in collaboration with Dr. Ruedi Aebersold, his colleague Dr. Tiannan Guo, and PBI scientists and engineers. The Aebersold Lab incorporates the PCT-HD System as an integral part of *PCT-SWATH* in their research efforts.