

Amgen Scientists Presents a Comparison of PCT & Microwave for the Digestion of Proteins at the Federation of Analytical Chemistry and Spectroscopy Societies (FACSS) Meeting September 28 - October 2, 2008, Reno, NV

PBI in the News

PBI Announces Research Agreement with the US Army Medical Research Institute of Infectious Diseases (USAMRIID)

Pressure BioSciences, Inc. announced that it has entered into a Cooperative Research and Development Agreement (CRADA) with the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). Researchers at USAMRIID have recently shown that the use of the Company's patented pressure cycling technology ("PCT") with patent-pending chemical reagents ("ProteoSolve-SB") has resulted in the simultaneous decontamination and extraction of macromolecules (DNA, RNA, proteins, lipids) as well as small molecules from samples containing infectious pathogens. The purpose of this CRADA is to adapt PCT into protocols for the development of medical countermeasures against dangerous pathogens that endanger the warfighter. The CRADA will allow scientists from PBI and USAMRIID to combine resources, experiences, and expertise to help achieve this important goal.

PCT Highlighted in Five Podium Presentations at the Fifth International Conference on High Pressure; PBI Introduces the Patent-pending "PCT Shredder" at the Meeting

Pressure BioSciences, Inc. announced that the Company's pressure cycling technology ("PCT") was highlighted in five separate podium presentations at the Fifth International Conference on High Pressure Bioscience and Biotechnology ("HPBB"), held from September 15-19, 2008 in San Diego, CA. The HPBB Conference was attended by nearly 100 high pressure research scientists from government, academic, and industry laboratories from around the world. PBI was one of several sponsors to support the meeting.

PBI also announced that it introduced its patent-pending "PCT Shredder" during the HPBB Conference, the third new PCT-dependent product that the Company has released over the past four months. The PCT Shredder was designed to help research scientists safely, rapidly, and conveniently disrupt very tough samples - such as ticks, muscle, and seeds - that require homogenization prior to PCT or other sample preparation methods.

Poster Presented by Amgen, Inc.

A Comparison of Methods for Efficient Digestion of Protein Therapeutics

ABSTRACT

Accomplishment of enzymatic proteolysis via conventional overnight digestion of a therapeutic monoclonal antibody with endoprotease Lys-C was compared to digestion using microwave assisted and pressure cycling technologies. Efficient digestion of proteins using both technologies has been reported in the literature; however there has been limited application to protein therapeutics.

The degree of digestion (number of missed cleavages), and time to achieve complete digestion were investigated for each technique, with conventional digestion used as the control for all experiments performed. Pressure cycling was shown to be most effective at achieving complete digestion in a short time, without unintended perturbation of the molecule. Microwave digestion did not achieve complete digestion and induced oxidation of methionine residues.

CONCLUSIONS

This study demonstrated that pressure cycling provided the most effective method for digesting monoclonal antibodies. Complete digestion can be obtained in a short period of time without inducing modifications such as methionine oxidation. While the microwave technique has established applicability in a proteomics setting, the more stringent requirements of the biopharmaceutical arena suggest limitations of the technique with respect for characterization of protein primary structure.

(Click here for the complete Poster)

Please Note: This poster is neither an explicit nor implicit endorsement of PCT by Amgen. It represents scientific data that are now in the public domain that were generated by scientists in an independent laboratory using PCT and other sample preparation methods

CALENDAR OF EVENTS

DISCOVERY 2 DIAGNOSTICS SAN DIEGO, CA OCTOBER 21-22, 2008	PLANT & ANIMAL GENOMICS SAN DIEGO, CA JANUARY 10-14, 2009
---	---

Pressure Enhanced Processing (PrEP) Focus

Proteolysis (Proteinase K)-PrEP: Pressure Enhanced Acceleration of Proteinase K Digestion of Tissue for Genomic DNA Extraction

Introduction

Genomic DNA extraction from tissues often includes digestion of the cellular proteins with Proteinase K before isolating genomic DNA from a prepared lysate to remove contaminating proteins from nucleic acid preparations. Protein digestion is the most time consuming step in most DNA isolation procedures. This lengthy digestion procedure may require 2-3 hours incubation at 45-55°C. In fact, some protocols call for as much as an overnight digestion to adequately remove contaminating protein. To accelerate this time consuming step and to achieve better digestion of proteins, Pressure BioSciences, Inc. (PBI) developed a method using pressure cycling technology (PCT) to enhance protein digestion by Proteinase K. Proteinase K is one of several enzymes enhanced by pressure. Others include trypsin, chymotrypsin and pepsin, Alcalase, Neutrase, Corolase 7089, Corolase PN-L, and papain. Here we show that tissue digestion by Proteinase K is accelerated under pressure, resulting in faster genomic DNA isolation both at 55°C and at ambient temperature in PBI's Pressure Cycling Technology Sample Preparation System (PCT SPS).

Results and Discussion

Results show that PCT enhances Proteinase K activity as indicated by both visual observation (dissolution of tissue pieces) and by increased DNA recovery at shorter digestion times. PCT in combination with Proteinase K resulted in more efficient recovery of DNA. Gel electrophoresis demonstrates similar patterns of genomic DNA in samples isolated at 20 and 35 kpsi and at atmospheric pressure, supporting the conclusion that the PCT protocol is gentle and does not lead to shearing of the genomic DNA (See Figure 1).

Results shown in Table 1 demonstrate that PCT is an effective tool for enhancing the activity of Proteinase K and for improving the efficiency of tissue digestion for genomic DNA isolation. Pressure cycling technology is an attractive option for rapid preparation of genomic DNA from various types of tissues by reducing the time-to-result and by increasing yield.

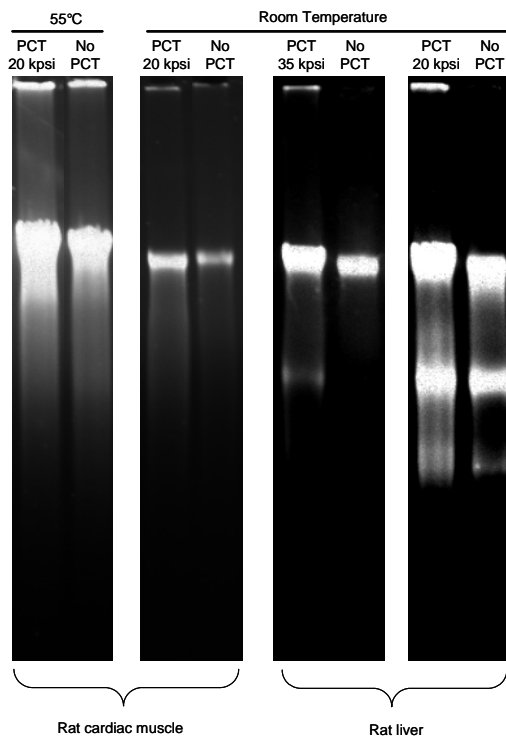


Figure 1. Genomic DNA Extracted from Rat Heart and Liver. Tissues were digested with Proteinase K with pressure cycling at 20 or 35 kpsi and compared to samples treated at atmospheric pressure. Digestion was performed either at ambient temperature or at 55°C.

Tissue	Time in Minutes	Temp	Pressure	Avg recovery: µg DNA per mg tissue	% of control*
Liver	130	Ambient	35 kpsi	0.66 (n=2)	228%
		Ambient	Ambient	0.29 (n=2)	
	90	Ambient	35 kpsi	1.09 (n=3)	279%
		Ambient	Ambient	0.39 (n=3)	
	100	Ambient	20 kpsi	1.47 (n=5)	155%
		Ambient	Ambient	0.95 (n=3)	
Heart Muscle	60	Ambient	20 kpsi	0.60 (n=2)	155%
		Ambient	Ambient	0.39 (n=2)	
	120	Ambient	20 kpsi	1.03 (n=2)	154%
		Ambient	Ambient	0.67 (n=2)	
	60	55°C	20 kpsi	3.95 (n=3)	153%
		Ambient	Ambient	2.59 (n=3)	

Table 1. Genomic DNA Extracted from Rat Liver and Heart Muscle by Proteinase K as a Function of Time, Temperature and Pressure. *Expressed as increase in DNA recovery (per mg tissue) versus control experiment performed at ambient pressure. Note that while DNA recovery in both control and PCT samples varies from experiment to experiment, the PCT enhanced DNA recovery is always higher than the corresponding control.