

## Key Advantages of Pressure Cycling Technology (PCT) in the Analysis of Glycoprotein-based Drugs and Drug Candidates Reported in Major Scientific Journal

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South Easton, MA, March 5, 2010 – Pressure BioSciences, Inc. (NASDAQ: PPIO) (“PBI” and the “Company”) today announced that a scientific article entitled “Rapid Release of N-Linked Glycans from Glycoproteins by Pressure Cycling Technology” has been published online in the journal, *Analytical Chemistry*. The article’s authors, from the Barnett Institute at Northeastern University (Boston, MA), reported on the ability of PCT to rapidly, effectively, and efficiently release sugar molecules (N-linked glycans) from certain proteins (glycoproteins) that are used in the development of biotherapeutic drugs.

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### Rapid Release of N-Linked Glycans from Glycoproteins by Pressure-Cycling Technology

**Zoltan Szabo, Andras Guttman, and Barry L. Karger\***  
*Barnett Institute, Northeastern University,  
Boston, Massachusetts 02115*

The standard, well-established sample preparation protocol to release N-linked glycans from glycoproteins for downstream analysis requires relatively long deglycosylation times (from several hours to overnight) and relatively high endoglycosidase concentration (from 1:250 to 1:500 enzyme:substrate molar ratio). In this paper, we significantly improve this standard protocol by the use of pressure-cycling technology (PCT) to increase the speed and decrease the relative amount of PNGase F during the release of N-linked glycans from denatured glycoproteins. With the application of pressure cycling from atmospheric to as high as 30 kpsi, >95% release of the asparagine-linked glycans from bovine ribonuclease B, human transferrin, and polyclonal human immunoglobulin was rapidly achieved in a few minutes using as low as 1:2500 enzyme:substrate molar ratio. The deglycosylation rate was first examined by SDS-PAGE at the protein level. The released glycans were then quantitated by capillary electrophoresis with laser induced fluorescence detection (CE-LIF). This new sample preparation protocol readily supports large-scale glycan analysis of biopharmaceuticals with rapid deglycosylation times.

The full text can be downloaded from:

<http://pubs.acs.org/doi/abs/10.1021/ac100098e>

### **PBI Now Sells IEF Reagent!**

ProteoSolve-IEF is designed to solubilize many types of proteins during sample preparation for a variety of analytical techniques, including isoelectric focusing (IEF) and gel-phase or solution-phase protein fractionation. This reagent contains powerful chaotropic agents and a zwitterionic detergent.

To order or to request additional information, please contact Matt Potter, V.P. of Sales at:

[mpotter@pressurebiosciences.com](mailto:mpotter@pressurebiosciences.com).

### **Scientists from University of North Texas Invited to Present on the Application of PCT in Forensics in Promega’s Tradeshow Booth at the 62nd Annual Scientific Meeting of AAFS**

Ms. Pam Marshall and Ms. Carey Davis, scientists from the University of North Texas Health Science Center at Forth Worth, Texas (“UNTHSC”), gave presentations at the American Academy of Forensic Sciences (AAFS) meeting in Seattle, WA, February 21-26, 2010 on the potential use of pressure cycling technology (PCT) in forensics. The scientists were invited by Promega to present UNTHSC findings in Promega’s booth on the tradeshow floor during the meeting. In two separate talks entitled **Pressure Cycling Technology (PCT) Applications for DNA Extractions from Bone Using the Promega Maxwell® 16**, data showed that PBI’s PCT Sample Preparation System (PCT SPS) used in combination with Promega’s Maxwell® 16 was capable of extracting high quality DNA from poor quality human bone to make a positive identification. Researchers interested in viewing Ms. Marshall’s and Ms. Davis’s presentation can do so by visiting Promega’s website at [www.mediastoreonline.com/promega](http://www.mediastoreonline.com/promega).

### CALENDAR OF EVENTS

<u><b>US HUPO 6<sup>TH</sup> ANNUAL CONFERENCE</b></u>	<u><b>BIOTHREAT AGENTS WORKSHOP2010</b></u>	<u><b>ABRF 2010</b></u>
Denver, CO	Charlotte, NC	Sacramento, CA
March 7-10, 2010	March 15-16, 2010	March 20-23, 2010

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Biotherapeutic drugs are viewed by many as the best current hope for the development of more effective treatments for acute, chronic, and life-threatening diseases, such as cancer, arthritis, and diabetes. The majority of these drugs - whether approved, in early development, or in clinical trials, are glycoproteins. In order to ensure quality, efficacy, safety, and biological function, a number of important tests are performed on glycoprotein-based biotherapeutic drugs and drug candidates, at every step of the development and approval process.

"Glycan (carbohydrate) analysis of biotherapeutic drugs provides essential information on quality, efficacy and safety; therefore, the biotech industry places great emphasis on these analyses," commented Professor Barry Karger, Director of the Barnett Institute and the senior author of the article. "Given the biological and therapeutic significance, rapid, robust, and accurate glycan analysis methods are of high interest."

Professor Karger continued: "We demonstrated that pressure cycling technology (PCT) can significantly improve the speed required for carbohydrate analysis, reducing the standard two hour/overnight enzymatic reaction to twenty minutes or less. PCT also required significantly less of an expensive enzyme needed in the analysis, reducing consumption by about 90%. In addition, PCT did not appear to lead to any apparent loss of sialic acid residues (a potential problem observed with other methods), and also offered the possibility of simultaneous processing of multiple samples, important for high throughput screening."

Dr. Nate Lawrence, Vice President of Marketing for PBI, commented: "We believe there are approximately 4,000 biotechnology companies worldwide and that many are investigating or developing glycoprotein-based drugs. We also believe that many of these companies are evaluating dozens to hundreds of different molecules as drug candidates, and that these molecules are tested multiple times throughout the development and approval process. We further believe that PCT can significantly improve the standard analysis protocol for these glycoprotein-based products, while concomitantly offering substantial financial savings due to a significantly reduced cost per test. Consequently, we are developing a focused plan to quickly and effectively reach these new potential customers of PCT."

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Dr. Alexander Lazarev, Vice President of R&D for PBI, said, "We believe these data are another important example of how enzymatic activity can be controlled by PCT. We also believe these results, together with previously reported data on the acceleration of enzymatic action in protein digestion, suggest that PCT may enhance the activity of other commercially important enzymes as well. Therefore, we believe that PCT may play a significant role in improving enzyme-based methods in other large and potentially lucrative fields of use within the biotechnology and pharmaceutical industries."

### **Forward Looking Statements**

Statements contained in this press release regarding the Company's intentions, hopes, beliefs, expectations, or predictions of the future are "forward-looking" statements within the meaning of the Private Securities Litigation Reform Act of 1995. Such forward looking statements include statements regarding the advantages of PCT in glycoprotein analysis, including the rapid, effective, and efficient release of sugar molecules; that biotherapeutic drugs are the best current hope for more effective treatments for cancer, arthritis, diabetes, and other diseases; that tests are run on glycoprotein-based drugs and drug candidates to ensure quality, efficacy, safety, and function, and that the biotech industry places great emphasis on such tests; that PCT can significantly improve the speed and decrease the cost of carbohydrate analysis, does not lead to sialic acid residue loss, and offers the potential for high throughput screening; that there are an estimated 4000 biotech companies worldwide and many are developing glycoprotein-based drugs; that the article's data indicate that PCT can control and enhance enzymatic action and the activity of other enzymes, and may improve enzyme-based methods in other fields within the biotech/pharma industries. These statements are based upon the Company's current expectations, forecasts, and assumptions that are subject to risks, uncertainties, and other factors that could cause actual outcomes and results to differ materially from those indicated by these forward looking statements. These risks, uncertainties, and other factors include, but are not limited to: possible difficulties, delays and additional costs in the implementation of the Company's strategies that may adversely affect the Company's commercialization of PCT and PCT-dependent products, including a plan to attract customers for glycoprotein analysis; changes in customer's needs and technological innovations; other scientists may not achieve the same PCT results reported by the Barnett Institute; the Company's sales force may not successfully sell the PCT product line because scientists may not perceive the advantages of PCT for glycoprotein analysis; the potential market for PCT for glycoprotein analysis may be substantially smaller than anticipated by Dr. Karger and the Company; and due to unforeseen costs or delays, the Company may require additional working capital to fund its operations before 2011. Additional risks and uncertainties that could cause actual results to differ materially from those indicated by these forward-looking statements are discussed under the heading "Risk Factors" in the Company's Annual Report on Form 10-K for the year ended December 31, 2008, and other reports filed by the Company from time to time with the SEC. The Company undertakes no obligation to update any of the information included in this release, except as otherwise required by law. Visit us at our website.

<http://www.pressurebiosciences.com>