

Pressure BioSciences Awarded \$649,000 Grant from the Department of Defense (“DoD”)

Non-dilutive Funding for the Development of an Automated, High-throughput, PCT Based System to Help in the Detection of Highly Pathogenic Organisms

Total of \$809,000 of Non-Dilutive Funding Awarded in Past Two Months

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South Easton, MA, October 13, 2011 – Pressure BioSciences, Inc. (NASDAQ: PBIO) (“PBI” and the “Company”) today announced that it has been awarded a \$649,498 SBIR Phase II grant (W81XWH-10-C-0175) from the U.S. Department of Defense (“DoD”). Entitled “Development of a Universal Method for Diagnostic Sample Inactivation, Extraction, and Enrichment of Pathogens in Arthropod Hosts of Military Importance”, this grant will help fund the development of an automated, high-throughput, high pressure system (instrument and consumables) for the safe and accurate processing of pathogenic organisms (viruses and bacteria). The system will be based on the Company’s patented, powerful, and enabling pressure cycling technology (“PCT”) platform.

Mr. Richard T. Schumacher, President and CEO of PBI, stated: “This grant award is very important and timely for PBI, as it provides \$649,000 in non-dilutive funding for the Company, payable over two years. Inclusive of our previously announced NIH SBIR I award, this brings the total of non-dilutive funding awarded PBI over the past two months to \$809,000.”

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PBI EVENTS

[Dr. Bradford Powell \(Founder and CSO, Cernomics Solutions\) will present a podium talk at the Integrating Sample Preparation 2011 Meeting Integrating Sample Preparation of Chemical & Biological Agents, Threats & Pathogens into Detection, Identification & Analysis Technologies & Devices entitled “A Need for Improved Sample Inactivation and Extraction Methods to Support Systems Biology Analysis” December 8-9, 2011 - Washington, DC](#)

Excerpt from Application Note: AN-00022

[Isolation of Functional Mitochondria from Whole Rat Brain Using a PBI Shredder and Pressure Cycling Technology \(PCT\)](#)

Introduction

Isolation of intact mitochondria from human and animal tissue is crucial for studies that focus on the elucidation of their function and dysfunction in conditions such as aging, diabetes and cancer. As potential drug targets, high quality functional mitochondrial isolates are important for drug screening studies [1]. Mitochondria isolation from solid tissue is usually carried out using labor-intensive homogenizer-based methods [2] that require extensive operator experience. Here we describe a semi-automated method to release mitochondria from solid rat brain tissue, using a PBI Shredder and PCT in place of traditional manual homogenization.

Results and Discussion

Here we demonstrate that high quality mitochondria can be extracted from rat brain tissue using The PBI *Shredder* and PCT. Similar results would be achieved using *The SHREDDER SG3* and PCT. This method is based on previous data that support the use of the *Shredder* and PCT for isolation of intact mitochondria from cell cultures [3], kidney and skeletal muscle [4], and lung tissue [5]. Total protein concentration of the final mitochondria preparations was 24 ± 7.4 mg/mL (n=4). Previous data generated using muscle tissue suggest that no improvement in yield is achieved when pressure is increased above 10,000 psi [4]; however, it is possible that with brain tissue, higher pressures could be beneficial.

The ability of isolated brain mitochondria to respond to Ca^{2+} is shown in Figure 2. These results confirm that rat brain mitochondria exposed to hydrostatic pressure cycling at 10,000 psi are intact and exhibit a normal response to Ca^{2+} overload.

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Mr. Schumacher continued: "The DoD award covers a substantial portion of the remaining development costs of an entirely new PCT-based instrument and consumables system, one that we believe will fill an existing need for a fully automated, high throughput, PCT-based system in the life sciences sample preparation market. We believe this is an estimated \$6 billion market, with approximately 80,000 laboratories and 500,000 researchers worldwide. We also believe that this new system has the potential to significantly expand PCT instrumentation and consumables sales into this large and growing market."

Dr. Edmund Y. Ting, Senior Vice President of Engineering for PBI, said: "The detection of dangerous pathogens in the field is essential to protect soldiers, emergency responders, and others against possible exposure to biological threat agents. Such detection requires the safe and rapid inactivation of infectious samples and the simultaneous extraction of biomolecules (DNA, RNA, proteins) from the pathogen, including highly dangerous bacteria such as anthrax. We believe that PCT is one of the best technology platforms available today that can concomitantly inactivate infectious samples and extract biomolecules from these pathogens, while leaving the sample in a form viable for accurate subsequent testing. Funds from this grant will be used to accelerate the development of this novel PCT-based system for the DoD."

Dr. Alexander V. Lazarev, Vice President of R&D for PBI, commented: "As we develop the DoD specific field use instrument, we plan to adapt the new PCT system for both research and clinical diagnostics laboratory use as well. We believe that we understand the issues involved and can make the modifications required to adapt the DoD field instrument for such use."

During the term of this grant, the Company will collaborate with scientists from both the U.S. Army Medical Research Institute of Infectious Diseases ("USAMRIID") and the University of Texas Medical Branch ("UTMB"). USAMRIID is the nation's premier research laboratory designed and dedicated to the discovery of medical solutions against biological threat agents. UTMB operates one of two National Biocontainment Laboratories constructed under grants awarded by the National Institutes of Health

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Application Note: AN-0022: Isolation of Functional Mitochondria from Whole Rat Brain Using a PBI Shredder and Pressure Cycling Technology (PCT):
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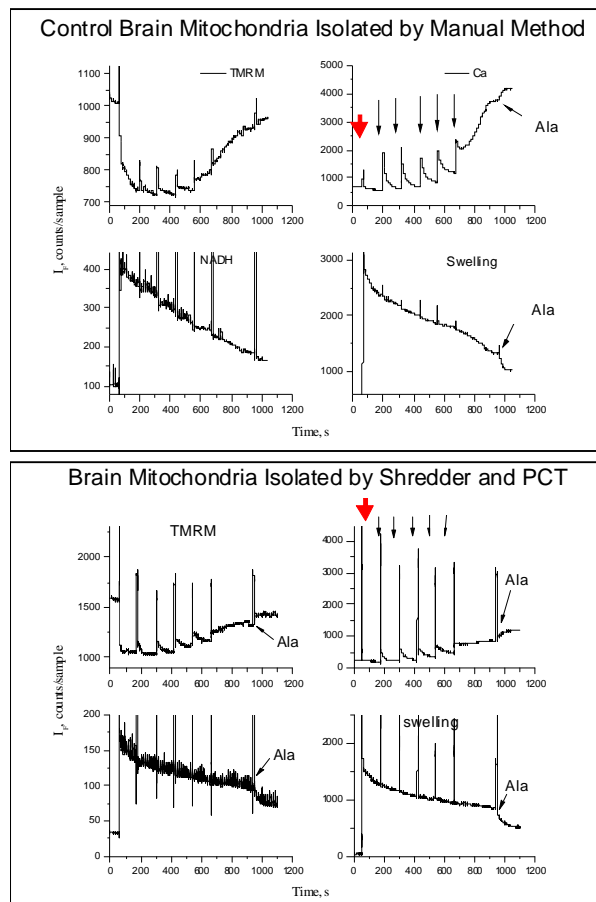


Figure 2. Mitochondria isolated by a conventional technique [6] (top panel) and Shredder/PCT method (bottom panel) demonstrate that mitochondria prepared by both methods are intact as assayed by their ability to respond to exogenous Ca²⁺. Addition of mitochondria to the sample chamber is indicated by the red arrow. Each Ca²⁺ addition is indicated by a black arrow (note that the scale on the Y-axes is not always the same in the 2 panels). The Ca²⁺-induced response was monitored by simultaneous four channel recording. Changes in membrane potential were monitored using TMRM. Ca²⁺-fluxes were monitored with CaGreen 5N, which increases its fluorescence when complexed with free extra-mitochondrial Ca²⁺. Oxidation of mitochondrial pyridine nucleotides was measured as a decrease in autofluorescence of NADH and NADPH. Swelling was detected as a decrease of light scattering.

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About Pressure BioSciences, Inc.

Pressure BioSciences, Inc. (PBI) (NASDAQ: PBIO) is focused on the development, marketing, and sale of proprietary laboratory instrumentation and associated consumables based on Pressure Cycling Technology ("PCT"). PCT is a patented, enabling technology platform with multiple applications in the estimated \$6 billion life sciences sample preparation market. PCT uses cycles of hydrostatic pressure between ambient and ultra-high levels to control bio-molecular interactions. PBI currently focuses its efforts on the development and sale of PCT-enhanced bio-molecule extraction and enzymatic digestion products for mass spectrometry, biomarker discovery, bio-therapeutics characterization, vaccine development, soil and plant biology, forensics, histology, and counter-bioterror applications.

Forward Looking Statements

Statements contained in this press release regarding PBI'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 expectation that the Company's development efforts funded by the Phase II grant from the DoD will be successful and will lead to the release of an entirely new PCT-based system for processing pathogenic organisms; the potential functionality and benefits of, and the market demand for, the new PCT-based system; the ability to significantly expand PCT instrument and consumables sales into the life sciences sample preparation market; that the life sciences sample preparation market is an estimated \$6 billion market, with approximately 80,000 labs and 500,000 researchers worldwide; that detection of pathogens in the field require the safe, rapid, and simultaneous inactivation and extraction of biomolecules from infectious samples; that PCT is one of best technologies that can safely inactivate and extract highly infectious pathogens while leaving the sample viable for analysis; that the new PCT-based system can be easily adapted for research and clinical diagnostics laboratory use; that a new 96-well plate format can be developed for the new PCT system, and that this format can be used to enable PCT-processed samples to be directly analyzed by current state-of-the-art analytical instrumentation through laboratory robotics and other automation; that funds from the new DoD grant will cover most of the development costs of the new PCT System; and that this new PCT system will fill an existing large and important need in the market. 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: due to unforeseen technical difficulties, the Company's development of the DoD specific field use instrument or the adaption of the DoD specific field use instrument for research and clinical diagnostics laboratory use, may be delayed or may not be successfully completed; possible difficulties or delays in the implementation of the Company's strategies that may adversely affect the Company's continued commercialization of its PCT-based product line; changes in customer's needs and technological innovations; the Company's sales force may not be successful in selling the Company's PCT product line because scientists may not perceive the advantages of PCT over other sample preparation methods; that the Company may not be successful in raising the additional capital necessary to fund the Company's operations beyond late November; if the NASDAQ Listing Qualifications Panel does not accept the Company's plan to regain compliance with the NASDAQ Listing Rule for minimum stockholder equity, the Company's common stock will be delisted from The NASDAQ Capital Market; and if actual operating costs are higher than anticipated, or revenues from product sales are less than anticipated, the Company may need additional capital sooner than expected. Given the uncertainty in the capital markets and the current status of the Company's product development and commercialization activities, there can be no assurance that the Company will secure the additional capital necessary to fund its operations beyond late November on acceptable terms, if at all. 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, 2010, 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.

For more information about PBI and this press release, please click on the following links:

<http://www.pressurebiosciences.com>

<http://bit.ly/p1zk9s>

Application Note: AN-00022: Isolation of Functional Mitochondria from Whole Rat Brain Using a PBI Shredder and Pressure Cycling Technology (PCT):
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Conclusions

Traditional manual methods for isolation of functional mitochondria from tissues rely heavily on operator training, experience and skill. Without considerable training, common mistakes such as tissue over-homogenization can result in damaged mitochondria and highly variable results. Here we describe a convenient method for isolation of intact and functional mitochondria from fresh rat brain tissue using a PBI Shredder and PCT. During shredding, the design of the FT 500-S PULSE Tube forces the homogenate through the holes of the Lysis Disk into the upper compartment. This simple but effective design significantly reduces the likelihood of sample over-homogenization. Following the shredding step, brief PCT treatment at 10,000 psi is used extract the intact mitochondria.

The PBI Shredders and PCT are physical tissue disruption/lysis methods that are widely applicable to many sample preparation needs. In the case of mitochondria isolation, the buffers described above can be readily replaced by other mitochondria isolation buffers depending on the needs and preferences of the user. As previously demonstrated with other tissue types, the mitochondria isolated using this convenient method can be used for proteomic as well as functional studies [3, 4]. This method is simple, easy to learn, and eliminates many of the problems of traditional manual mitochondria preparation methods.

References

- [1] Armstrong (2007) Brit. J. Pharm. 151, 1154-1165
- [2] Rasmussen et al., (1997) Anal. Biochem. 252:153-159.
- [3] Gross et al. (2008) Biotechniques 45(1):99-100
- [4] Gross et al. (2011) Anal. Biochem. *In Press*
- [5] Application Note AN-00021: [Isolation of Mitochondria from Rat Lung Using The PCT Shredder and Pressure Cycling Technology \(PCT\)](#)
- [6] Stavrovskaya et al. (2010) Free Rad. Biol. & Med. 49 : 567-579

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