

## Improved Protein Recovery from Muscle Tissue Using *The PCT Shredder*<sup>™</sup> and Pressure Cycling Technology (PCT): Efficient Single Tube Sample Disruption and Extraction

### Introduction

Rapid, efficient and reproducible extraction of proteins from muscle is crucial for proteomic analysis and for studies of various conditions such as aging [1], hypertension [2], hypoxia and reperfusion-induced damage [3]. Protein extraction from tough tissues such as cardiac and skeletal muscle generally requires extensive mechanical or chemical disruption of the sample to adequately analyze its proteome. Mortar and pestle grinding, pulverization in liquid nitrogen or homogenization with a dounce or polytron homogenizer are some of the classical methods that can be used for the disruption of muscle tissue [1, 2, 3, and 4]. However, these manual methods are inherently inconsistent, time consuming and potentially hazardous. Here we describe a system for efficient tissue disruption and protein extraction from bovine cardiac muscle, in a single sample processing tube, using *The PCT Shredder* and the Pressure Cycling Technology Sample Preparation System (PCT SPS). Mechanical tissue disruption with *The PCT Shredder* is rapid and convenient; and, when it is combined with the power of PCT, it is an efficient and reproducible method to prepare whole tissue lysates from tough samples such as cardiac and skeletal muscle.

### Pressure Cycling Technology (PCT)

In the Pressure Cycling Technology Sample Preparation System (PCT SPS) hydrostatic pressure is rapidly cycled between ambient and ultra high levels (up to 45,000 psi) to control biomolecular interactions [5]. High hydrostatic pressure acts preferentially on the compressible components of the sample, such cell membranes, resulting in cell lysis and the release of intracellular contents. The PCT SPS can be used to disrupt animal and plant tissues, cells, cellular structures and microbes to extract nucleic acids, proteins and lipids. The system is comprised of a small, semi-automated bench-top instrument (Barocycler NEP3229 or the NEP2320) used in combination with single-use sample processing containers (PULSE Tubes). PCT in the presence of suitable extraction reagents, such as DNAzol<sup>®</sup>, results in isolation of intact DNA for genomic analysis and other applications.

### The PCT Shredder

The PCT Shredder is designed to mechanically disrupt the tissue in order to enhance extraction of tough, fibrous and other difficult-to-disrupt biological materials such as certain animal and plant tissues [6]. The PCT Shredder is used to rapidly grind the sample directly in a specially designed Shredder PULSE Tube to increase the tissue surface area and to improve cell lysis prior to treatment by PCT in order to extract nucleic acids, proteins, lipids and other cellular contents. Since shredding and PCT are done in the same tube, loss of sample or the likelihood of cross contamination is significantly reduced as compared to other processing methods.

### Materials and Methods

Thawed bovine cardiac muscle tissue (100 mg per sample) was processed in a Shredder PULSE Tube using *The PCT Shredder* as described in the User Manual (Pressure BioSciences, Inc.) in 0.4-0.5 mL of either Phosphate Buffered Saline (PBS) or ProteoSolve-IEF Reagent supplemented with 50 mM DTT. Subsequently, the cap to the Shredder PULSE Tube was removed and the sample volume was

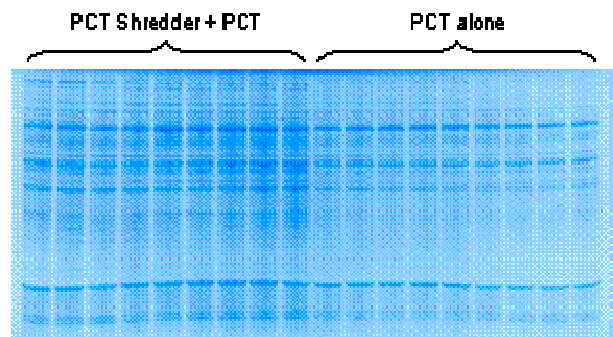


Figure 1. Total Protein from Cardiac Muscle Visualized on an SDS-PAGE Gel Stained with Coomassie Blue. All samples were extracted in physiological buffer (PBS). The 9 samples on the left were disrupted by *The PCT Shredder* prior to extraction by PCT. The 9 control samples on the right were extracted by PCT alone. Pooled Bradford protein assay results are shown in Figure 2.

brought up to 1.4 mL with additional extraction buffer. The Shredder PULSE Tube was then re-capped with the PULSE Tube Cap and subjected to PCT under the following conditions: 35,000 psi held for 20 seconds, followed by atmospheric pressure held for 10-20 seconds; repeated for 20 cycles. All extractions were performed at ambient temperature. Following protein extraction in either PBS or in ProteoSolve-IEF, samples were centrifuged to pellet any residual solid debris and the clarified supernatant was used for protein quantification by Bradford protein assays (Bio-Rad). Protein assay results from replicate samples were averaged. Total protein from samples extracted in PBS was visualized by SDS-PAGE using 8-16% Criterion gels (Bio-Rad).

### Results and Discussion

Protein recovery from samples processed by The PCT Shredder in PBS were 3.2-fold greater than samples processed by PCT alone (35.3 +/- 1.9 vs. 11.0 +/- 6.1 µg protein per mg of tissue). A similar improvement in protein recovery was observed in samples extracted in ProteoSolve-IEF reagent. The PCT Shredder increased total protein recovery in IEF buffer by 2.5-fold (71.7 +/- 5.9 vs. 28.7 +/- 7.6 µg proteins per mg of tissue). These data indicate that although buffer choice has a pronounced impact on protein recovery (Figure 2), use of The PCT Shredder in combination with PCT can improve protein extraction in various buffers and may allow the researcher to recover sufficient protein even in very mild buffer such as PBS. Data from both protein assays and SDS-PAGE analysis confirm that total protein recovery is significantly higher when muscle tissue is disrupted with The PCT Shredder prior to protein extraction by PCT. These results show that the preparation of crude total protein lysate from tough and fibrous tissue such as muscle is facilitated by the use of The PCT Shredder.

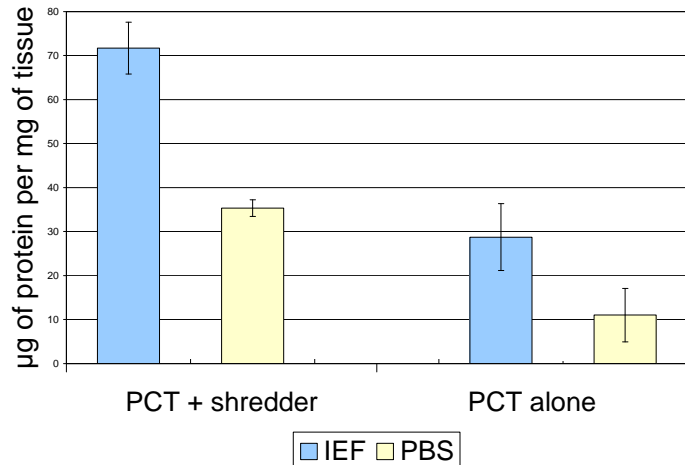


Figure 2. Protein Quantification by Bradford Assay. Total protein was extracted in PBS (n=9 per group) or ProteoSolve-IEF reagent (n=6 per group). Results are expressed as micrograms of recovered protein per milligram of tissue sample.

### Conclusions

The combination of mechanical sample disruption by The PCT Shredder followed by extraction using pressure cycling technology (PCT) is an effective and safe method for isolation of protein from tough tissues such as cardiac and skeletal muscle. Rapid, efficient and reproducible extraction of proteins can be obtained for proteomic analyses, even with very mild buffers such as PBS. In addition, since disruption and extraction are performed in the same container (Shredder PULSE Tube), sample recovery is enhanced, and the likelihood of cross-contamination is minimized.

### References

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