

Extraction of Proteins and RNA from Small Solid Tissue Samples Using PCT and FT 500-ND PULSE Tubes

Introduction

Efficient, reproducible and rapid tissue disruption and extraction of biomolecules are prerequisite for many biological applications. Solid tissues, especially tough or fibrous ones like muscle, generally require extensive mechanical disruption prior to extraction. Mortar and pestle grinding, pulverization in liquid nitrogen or homogenization with a dounce or polytron homogenizer are some of the classical methods that have been used for tissue disruption. However, these manual methods are often inconsistent, time consuming and potentially hazardous. In addition, due to the amount of sample loss inherent in these methods, they are often unsuitable for use with small samples in the 10 μ g size range.

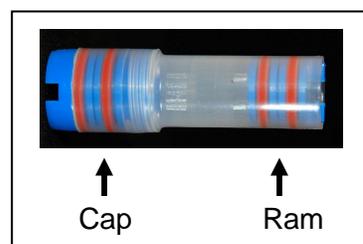
Here we describe a system for efficient tissue disruption and extraction of protein or RNA from solid tissues using the Pressure Cycling Technology Sample Preparation System (PCT SPS) and the FT 500-ND PULSE Tubes. Pressurization of small sample volumes in these tubes causes repeated compression of the sample tissue between the PULSE Tube Cap and Ram. This high-pressure mechanical tissue disruption, combined with the power of pressure cycling technology (PCT), is an efficient and reproducible method to prepare whole tissue lysates from solid tissue samples for extraction of proteins or nucleic acids.

PCT Sample Preparation System (PCT SPS)

The Pressure Cycling Technology Sample Preparation System (PCT SPS) uses rapid cycles of hydrostatic pressure between ambient and ultra high levels to control biomolecular interactions. The PCT SPS can be used to disrupt tissues, cells, and cellular structures to extract proteins and nucleic acids as well as lipids and small molecules [1, 2]. High hydrostatic pressure acts preferentially on the more compressible constituents of the sample, this selective energy distribution results in destabilization of molecular interactions but not in the disruption of covalent bonds. In addition, the PCT SPS can be used to accelerate enzymatic reactions such as trypsin and proteinase K digestion.

The PCT SPS uses a microprocessor-controlled bench-top instrument (Barocycler NEP3229 or the NEP2320) in combination with single-use sample processing containers. These disposable sample containers are available in several configurations. For extraction of solid samples in the 50-250 mg range (~1.4 mL per sample), the FT500 PULSE Tube is used. Applications requiring *The PCT Shredder* use the FT 500-SR PULSE Tubes. The diskless FT 500-ND PULSE Tube (Figure 1) can be used for extracting liquid or suspension samples such as bacteria, or small-scale solid tissue samples such as biopsies. For small-scale (50-150 μ L per sample), higher throughput applications, the system can also be used with the PCT MicroTubes. Here we describe the use of the FT 500-ND PULSE Tube for extraction of proteins and RNA from small samples of rat liver and skeletal muscle.

Figure 1. FT 500-ND PULSE Tube. The maximum volume per tube is 1.5 mL for liquid and suspension samples. For small scale tissue extraction, the optimal total sample volume is 150-200 μ L.



Materials and Methods

Preparation of FT 500-ND PULSE Tubes

The Ram was inserted into the FT500-ND PULSE Tube and pushed until the ram surface was ~4mm from the cap threads. With small sample volumes (i.e., \leq 200 μ L), this configuration permits the ram to “mash” the tissue against the cap at every pressurization cycle. The tissue was placed into the PULSE Tube along with 140 μ L of extraction reagent. The tube was then sealed with the PULSE Tube cap and subjected to pressure cycling as described below.

PCT Treatment

All samples were extracted by PCT in an NEP 3229 or NEP2320 Barocycler. Extraction conditions were as follows: 35,000 psi held for 20 seconds, atmospheric pressure held for 10 seconds; repeated for 20 cycles. All PCT extractions were performed at ambient temperature.

Extraction of Protein from Tissue

Frozen rat liver or skeletal muscle tissue (~10 mg per sample) was placed into FT 500-ND PULSE Tubes with 140 μ L of ProteoSolve-IEF Reagent. PULSE Tubes were capped and PCT was carried out as described above. Following PCT extraction, samples were transferred to microcentrifuge tubes and centrifuged to pellet residual solid debris. The clarified supernatant was used for protein quantification by Bradford protein assays (Bio-Rad). Protein assay results from replicate samples (n=5) were averaged. Control extractions (n=5) were performed in a 1 mL Potter-Elvehjem tissue grinder using the same tissue mass and extraction reagent volumes as above. The tissue grinder was thoroughly washed with distilled water between samples.

RNA Extraction

Rat frozen liver tissue (~10 mg per sample) was placed into FT 500-ND PULSE Tubes with 140 μ L of Trizol Reagent (Invitrogen). PCT was carried out as described above. Following PCT extraction, samples were transferred to microcentrifuge tubes. RNA was isolated from the Trizol Reagent as described by the manufacturer; except that volumes were scaled down to match the starting Trizol volume (140 μ L). Resulting RNA pellets were dissolved in 20 μ L of DEPC-treated water. RNA recovery was quantified by NanoDrop (Thermo Fisher). Results from replicate samples (n=3) were averaged. RNA quality was estimated by running the RNA on agarose gels. Control large-scale extractions (n=3) were performed in standard FT 500 PULSE Tubes by extracting ~100 mg of sample in 1.4 mL of Trizol Reagent using the same PCT conditions as described above. Final RNA pellets from the control extractions were dissolved in 100 μ L of DEPC-treated water

Results and Discussion

Here we compare a standard tissue disruption method to our new scaled-down method that utilizes PCT and FT 500-ND PULSE Tubes for tissue disruption and extraction of small solid tissue samples. Tissue disruption by pressure cycling in FT 500-ND PULSE Tubes results in efficient protein extraction from soft tissues such as liver and hard tissues such as muscle (Figure 2). The protein recovery (μ g of extracted protein per mg of starting material) of the new scaled-down protocol is within the range of control samples processed using a standard grinder method.

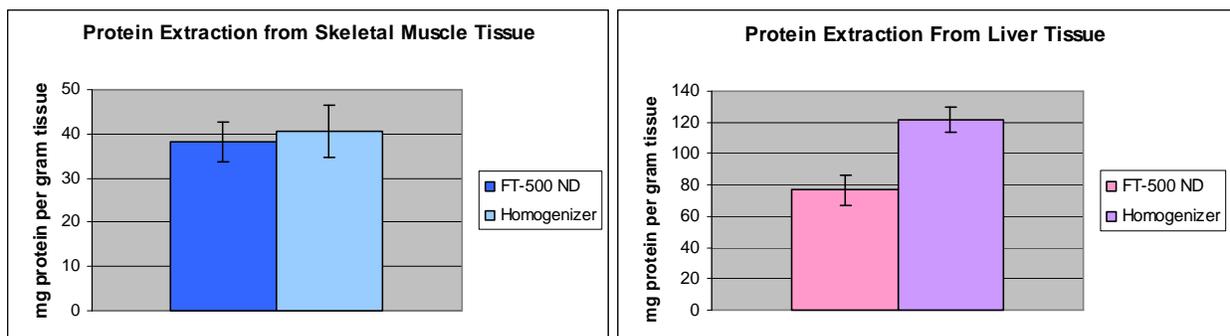


Figure 2. Protein recovery from skeletal muscle and liver tissue. Protein extraction was performed as described above. Yield is expressed as milligrams of protein recovered per gram of wet tissue weight. Error bars indicate standard deviation.

This new, scaled-down method can also be used for RNA extraction by simply replacing the ProteoSolve-IEF reagent with one that is designed for RNA isolation (e.g., Trizol Reagent). The results in Figure 3 demonstrate that efficient RNA recovery from small samples can be obtained using the FT 500-ND Tubes.

The agarose gels confirm that RNA extracted from small samples in the FT 500-ND Tubes is of comparable quality to RNA extracted using the standard PCT protocol in regular FT 500 Tubes with a lysis disk.

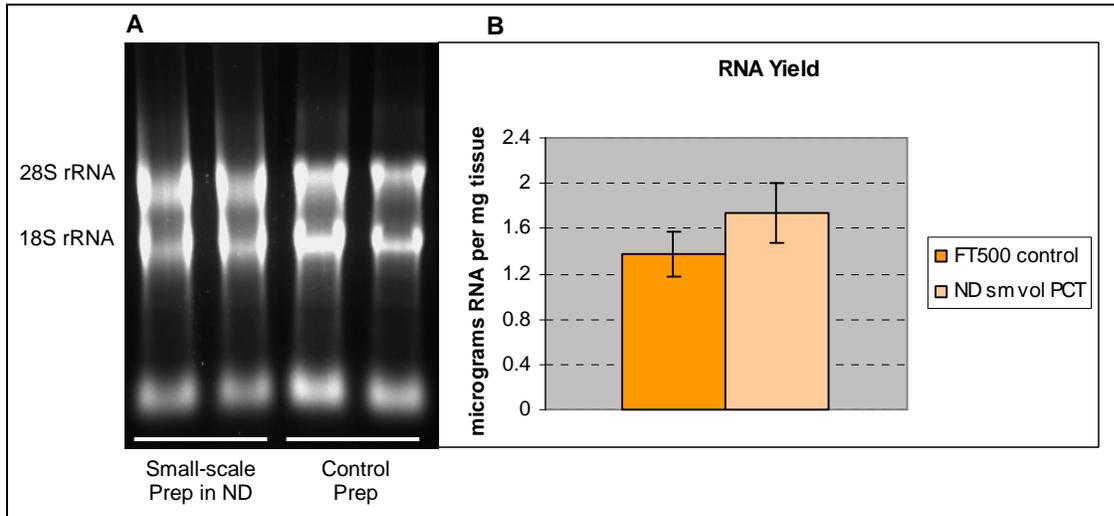


Figure 3. RNA extraction by PCT from liver tissue using the small-scale protocol in FT 500-ND PULSE Tubes (10 mg tissue per sample) compared to the standard method in regular FT 500 Tubes (100 mg tissue per sample). **A.** Agarose gel electrophoresis confirms that RNA extracted by the scaled-down method is intact and comparable to control. **B.** RNA yield is comparable by both methods (n=3). Error bars indicate standard deviation.

Conclusions

Conventional methods for tissue homogenization and extraction typically rely upon mechanical disruption by Dounce or other homogenizers, or mortar and pestle grinding. These methods may not be optimal for use with small samples due to the large amount of loss during sample processing and transfer. The method described here is a single-tube protocol that reduces the likelihood of sample loss. In addition, since the PULSE Tubes are disposable, they minimize the chances of sample cross-contamination and reduce processing time by eliminating the need to wash and dry grinders, mortars, pestles or other equipment. The use of disposable sample processing containers is especially convenient when working with RNA, because they also minimize the likelihood of RNA degradation by contaminating RNases. The new scaled-down method described here can also be used for extraction of genomic DNA from tissue by using a reagent appropriate for DNA isolation.

References

- [1] Schumacher RT *et al.* (2002). *Am. Laboratory* 34, 38-43.
- [2] Tao F, *et al.* (2007). *Proc. 4th Int. Conf. On High Pressure Biosci. and Biotech.* 1, 166-173.