Fast and Efficient Protein Extraction in MicroTubes Using PCT µPestles

Introduction

Homogenization and extraction from small tissue samples (<5.0 mg) requires scaled-down protocols that minimize sample dilution and loss. Here we describe a simple and rapid method for the efficient extraction of protein from small solid tissue samples using pressure cycling technology (PCT) and PCT µPestles.

PCT µPestles used in combination with PCT MicroTubes and PCT are designed for processing 0.5-5 mg (optimum ~2.0 mg) of soft solid tissue in 20-30 µl of extraction reagent for effective tissue disruption and cell lysis. To maximize protein recovery, the µPestles are made of PTFE (aka Teflon® by DuPont) and the MicroTubes are made of FEP, an inert, non-wetting Teflon-like material that has very low protein binding properties. The PCT µPestles and MicroTubes, together with any Barocycler model, comprise the PCT Sample Preparation System (PCT SPS). The PCT SPS provides a fast, safe, and efficient means of protein extraction from small solid samples such as soft animal tissues or biopsies.

Figure 1

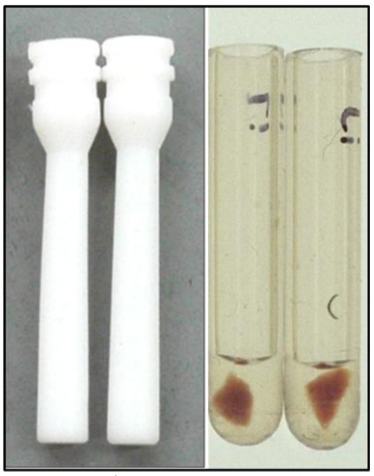

Figure 1. *Appearance of PCT µPestles and MicroTubes.* MicroTubes are shown with ~3mg of rat liver tissue and 30 µl of extraction reagent. Once inserted into the MicroTubes, the tight-fitting µPestles force the tissue into the small amount of space available. During pressure cycling, the compressible tube is repeatedly forced against the incompressible µPestle, resulting in efficient tissue homogenization and cell lysis.

Results and Discussion

To optimize extraction using the new PCT µPestles, experiments were performed to determine the optimal mass of tissue per sample. Protein was extracted from various tissues using 2, 5 or 10 mg of tissue per tube, with 30 µl of extraction reagent. The data suggest that for best results, samples should be ~2 mg (Figure 2). Larger samples (5 and 10mg) resulted in less efficient protein extraction and lower yields, most likely as a result of protein saturation in the extraction reagent.

The effect of pressure level was evaluated by comparing samples processed at ambient pressure (i.e., no pressure cycling), 10,000 psi (70 MPa/700 bar) and 35,000 psi (240 MPa/2400 bar). Results show that yields were very low in samples processed without PCT, while at 10,000 psi yields were slightly lower than at 35,000 psi. Therefore, 35,000 psi was used for all subsequent tests.

The efficiency of extraction was also tested using different numbers of pressure cycles. Samples were processed using 10, 60 or 99 pressure cycles. Results show that protein yield is optimal at ~60 cycles (Figure 3) when pressure cycling is performed at 35,000 psi. Using fewer cycles results in lower protein yield, especially with tougher tissue such as lung. Conversely,

running too many cycles seems to have a slightly adverse effect, perhaps due to protein aggregation that can occur during prolonged pressure cycling (the extent of aggregation depends on the extraction reagent being used. Samples in milder reagents, such as PBS or water, may exhibit more aggregation than samples in detergent-containing reagents). Optimal number of pressure cycles may vary depending on the tissue type as well as the extraction reagent. Sixty (60) cycles should be enough for maximal protein yield in most applications, but not so high as to induce significant aggregation.

Figure 2

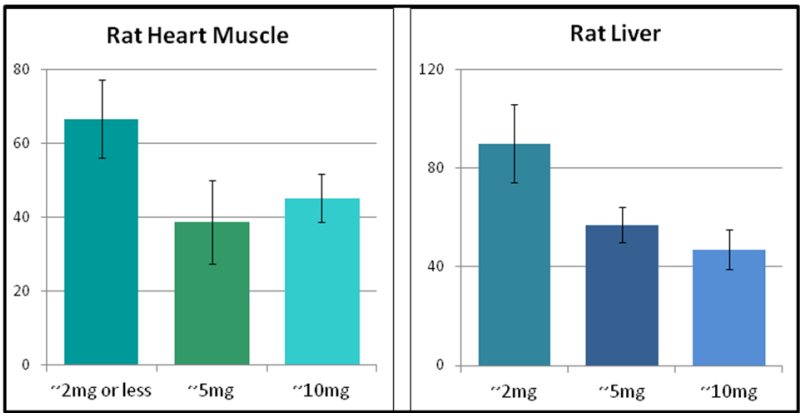

Figure 2. *Effect of tissue mass on the efficiency of protein extraction.* Rat heart muscle and liver tissue were extracted using the PCT µPestle method. Tissue pieces of 2, 5 or 10 mg were placed in MicroTubes with 30 µl of extraction reagent and processed at 35,000 psi for 60 cycles. Protein concentration in the lysate was measured and the final yield was calculated as µg protein per mg of tissue. Error bars indicate standard deviation. n=6 per group.

Figure 3

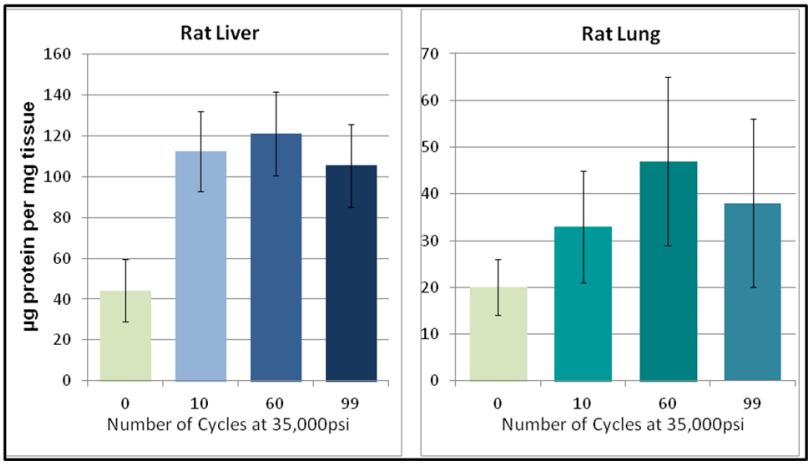

Figure 3. *Effect of increasing number of pressure cycles on protein yield.* Rat lung and liver tissue were extracted using the PCT µPestle method. All tissue pieces were in the range of 0.5- 2 mg. Protein concentration in the lysate was measured and the final yield was calculated as µg protein per mg of tissue. Error bars indicate standard deviation. n=10 per group.

Figure 4

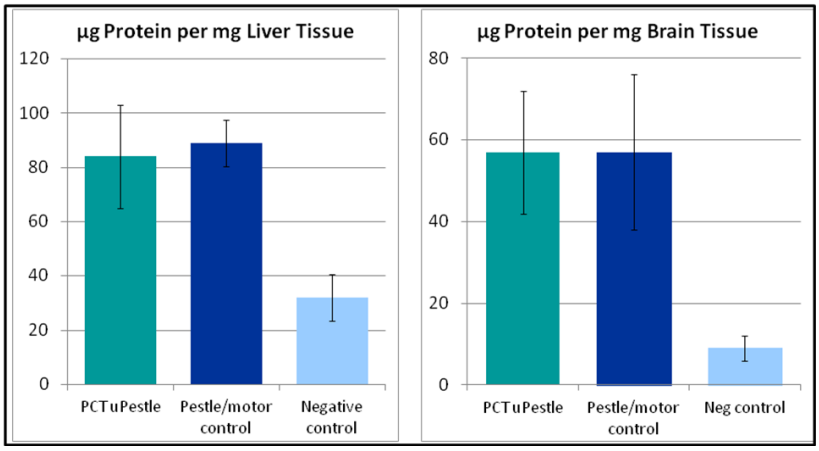

Figure 4. *Comparison between the PCT µPestle method and a motorized pestle grinder (pestle/motor control).* All tissue pieces were in the range of ~0.5-2 mg. Protein concentration in the lysate was measured and the final yield was calculated as µg protein per mg of tissue. Negative controls were placed into extraction reagent, but were not disrupted. Error bars indicate standard deviation. n=6 per group.

The PCT μPestle method was compared to a commonly used small-scale method that utilizes a hand-held motorized Kontes Pellet Pestle Grinder. This method depends in a tight fit between the pestle and the walls of the container, and is performed manually, one sample at a time. Protein yield was compared between the two methods using two different tissue types. Results indicate that the yield obtained using the PCT μPestle method is comparable to the grinder (Figure 4). The benefit of the PCT μPestle method is that once the samples are placed into the tubes, the processing is hands-off and can be performed with many samples in parallel. The Barocycler NEP 2320 can accommodate and process 12 such samples, while the Barocycler NEP 3229 can handle up to 48 samples at once. In addition, the PCT μPestle method is performed in closed MicroTubes, reducing the likelihood of sample loss, cross-contamination and exposure of the user to potentially hazardous or infectious materials.

Materials and Methods

Tissue pieces (0.5-2.0 mg, unless indicated otherwise) were rinsed in PBS to remove surface blood, blotted dry, individually weighed, and transferred with clean forceps to MicroTubes. ProteoSolve-IEF extraction reagent, 30 μl per sample, was added and the tubes were capped with the PCT μPestles. The samples were disrupted in the NEP 2320 Barocycler at ambient temperature by pressure cycling at 35,000 psi (unless indicated otherwise) using 10-99 pressure cycles. Each cycle consisted of 20 sec at 35,000 psi and 15 sec at ambient pressure. Samples were centrifuged directly in MicroTubes at 16,000g for 10 mins to pellet debris. For control extractions, the tissue pieces were disrupted with a hand-held motorized Kontes Pellet Pestle Grinder with matching tubes and pestles (Kimble Chase), using the same tissue mass and extraction reagent volume as used in the MicroTubes. Protein concentration in the lysate was measured by Bradford assay. For all samples the yield is expressed as μg of protein extracted per mg of tissue starting mass.

Summary

The PCT μPestle method is an effective tool for rapid and convenient disruption and extraction of small solid tissue samples, such as liver, brain, lung, etc. The μPestles, used with PBI Barocyclers, allow for significantly reduced hands-on time and increased throughput, compared to standard manual homogenization. The results presented here provide a starting point for protocol optimization. Excellent results are achieved when samples are less than 5 mg, (ideally in the 2mg range) and pressure cycling is performed for ~60 cycles (fewer cycles are likely to be sufficient for many applications) at 35,000 psi (240 MPa/2.4 kbar).

Patent Pending

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