Total Protein Extraction from Small Tissue Samples Using PCT MicroTubes and the ProteoSolve-SB Kit

Extraction of total proteins from tissue has generally been limited by the poor solubility of many proteins in traditional extraction buffers. This has been especially true for lipid-rich samples such as adipose tissue, but also for many other types of samples. Traditional detergent-based sample preparation methods may not adequately dissociate all proteins, especially hydrophobic proteins, which may be tightly associated with membrane lipids. Isolation of these proteins is often very inefficient, because the bulk of membrane proteins are often discarded in the insoluble fraction after extraction. As a result, proteomic analysis of tissues is often biased toward the more soluble proteins. We have previously described a method for efficient extraction of proteins from samples of a variety of mammalian tissues, using pressure cycling technology (PCT) and the novel chemistry of Pressure BioSciences' ProteoSolve-SB kit. Here we show that by using the new PCT MicroTubes, the ProteoSolve-SB protocol may be scaled down for use with tissue samples in the 10-20 mg size range. This scaled-down method is compatible with biopsy-size tissue samples.

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. High hydrostatic pressure acts preferentially on the more compressible constituents of the sample, and 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 NEP2320 or the NEP3229) in combination with single-use sample processing containers. For relatively large amounts of tissue (up to 500 mg), the PCT SPS uses FT500 PULSE Tubes to process 1-3 samples at a time. For smaller amounts of tissue (50-150 µL per sample), the PCT SPS uses PCT MicroTubes (Figure 1), to process 12-48 samples at a time. MicroTubes are made from highly inert plastic, ideal for the extraction of proteins.

Figure 1. PCT MicroTubes. MicroTubes are shown with 150 µL MicroCaps and cartridge that holds up to 8 samples. Up to 6 cartridges (48 samples) fit into the NEP3229 at one time. MicroTubes are also available with 100 µL and 50 µL caps for smaller sample volumes.

ProteoSolve-SB and PCT

The ProteoSolve-SB kit utilizes a detergent-free extraction reagent to extract proteins from a variety of tissues, including lipid rich samples such as adipose tissue and protease-rich tissues such as pancreas. This protocol exploits the synergistic combination of sample disruption by PCT and the unique ProteoSolve-SB reagent system that partitions extracted proteins and lipids into separate fractions (Figure 2).

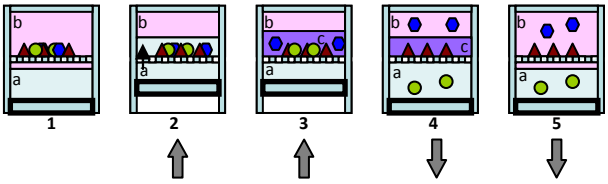

Figure 2. Diagram of sample disruption, extraction and fractionation by PCT in the ProteoSolve-SB kit. Panel **1**: Starting condition at atmospheric pressure. Two solvents "a" and "b" are immiscible at atmospheric pressure. Panels **2** and **3**: When high pressure is applied (up arrows), the solid sample is disrupted and the two solvents mix, forming a transient solvent "c". Panels **4** and **5**: When pressure is released (down arrows), the sample components fractionate by solubility and solvents "a" and "b" separate.

Methods

For small-scale PCT extraction using ProteoSolve-SB kit: 10 mg of rat liver, 20 mg of rat adipose or 20 mg of mouse brain was placed into individual PCT MicroTubes. 100µL of ProteoSolve-SB Reagent A and 35 µL of Reagent B were added to bring the volume ~150 µL. Tubes were capped with 150 µL PCT MicroCaps. Control samples were scaled up 10-fold to keep the

mass-to-volume ratio constant in all samples. 100 mg of rat liver, 200 mg of rat adipose or 200 mg of mouse brain was placed into an FT-500 PULSE Tube. 1.0 mL of ProteoSolve-SB Reagent A and 350-400 µL of Reagent B were added to bring the total volume to 1.4 mL per PULSE Tube.

All samples were thoroughly vortexed for 10-20 seconds before and after PCT. Pressure cycling was carried out in a Barocycler NEP3229 or NEP2320 for 20 cycles at ambient temperature. Each cycle consisted of 20 seconds at 35,000 psi followed by 10 seconds at atmospheric pressure. Following PCT, the large-scale samples were transferred from the PULSE Tubes to 2 mL tubes. The small-scale samples were left in the MicroTubes, which were placed into 2 mL tubes for centrifugation: e.g. the entire MicroTube was placed into a 2 mL tube in order to fit into the centrifuge rotor. All samples were centrifuged for 15 minutes at 12,000xg to separate the upper lipid phase from the protein-containing lower phase. The solubilized protein fractions were transferred to clean tubes and two 10 µL aliquots from each sample were dried by evaporation in a fume hood. One replicate aliquot was used for protein quantification by Bradford assay. The other was dissolved in 1X Laemmli buffer for SDS-PAGE.

Results and Discussion

Previous methods for protein extraction from tissues for proteomic analysis have relied upon mechanical disruption such as dounce or other homogenizers, or mortar and pestle grinding. These methods are extremely inefficient when used with small samples, principally due to the large amount of loss during sample disruption and transfer. In addition, most of these methods rely upon detergents such as SDS, Triton X-100 or CHAPS to solubilize membranes and extract proteins. Since many downstream analysis methods are incompatible with detergents, extensive sample clean-up is often required, potentially leading to significant protein loss.

Pressure-mediated extraction in combination with the ProteoSolve-SB kit has been shown to be an efficient method for protein extraction from lipid-rich adipose tissue [1] as well a variety of other tissues [2]. ProteoSolve-SB is detergent-free and can be efficiently removed from the extracted sample by evaporation or precipitation (precipitation reagent is provided in the kit).

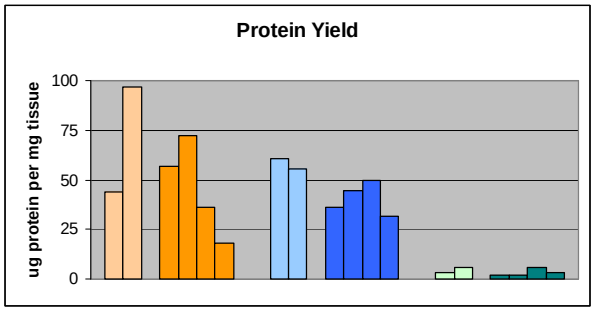

Figure 3. Protein Yields**.** Protein recovery is expressed as micrograms extracted protein per milligram tissue. Samples: Liver: PULSE Tube control (light orange), n=2; Liver: MicroTube samples (dark orange), n=4; Brain PULSE Tube control (light blue), n=2; Brain MicroTube samples (dark blue), n=4; Adipose PULSE Tube control (light green), n=2; Adipose MicroTube samples (dark green), n=4.

Data show that tissue disruption by pressure cycling in PCT MicroTubes, coupled with protein extraction using the detergent-free ProteoSolve-SB kit, results in adequate yield of proteins for analysis from a variety of small tissue samples. The protein recovery (µg of extracted protein per mg of starting material) of this protocol is within the range of control samples processed using the standard large-scale extraction methods (Figures 3 and 4).

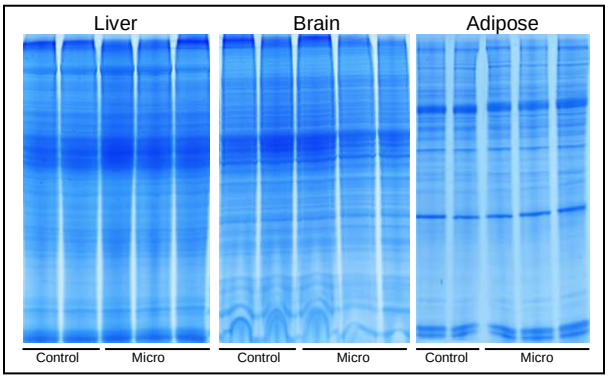

Figure 4. SDS-PAGE Analysis. After extraction, aliquots of the protein-containing fraction were run on 8-16% Tris-glycine polyacrylamide gradient gels. Control samples were extracted in 1.4 mL PULSE Tubes, Micro samples were extracted in PCT MicroTubes.

These data confirm that the combination of the PCT SPS, ProteoSolve-SB and MicroTubes provides for large-scale (up to 48 samples) screening of the proteome of a wide variety of tissues, including adipose tissue.

References

[1] Lazarev, AV., *et al*., 2007. J. Am. Soc. Mass Spect. p. 93S.
[2] Gross, V., *et al*., 2008. J. Biomol. Tech. 19:189-99.

v1.051610