

Improved Extraction of Biomolecules from Plants, Animals, and Fungi with The PCT Shredder

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ABSTRACT

The high content of fibrous material in many plant samples, as well as the presence of rigid cell walls, complicates the extraction of DNA and other biomolecules from plant tissues. To release target analytes, plant samples often require extensive and time consuming sample disruption, often accomplished by grinding with a mortar and pestle or by homogenizing with glass or metal beads. Such methods are often inefficient and may even be deleterious to the biomolecules. Here we describe a system for the efficient extraction of biomolecules from plants, animals, and fungi using *The PCT Shredder* and the Pressure Cycling Technology Sample Preparation System (PCT SPS). Initial disruption of plant tissue with *The PCT Shredder* followed by DNA extraction by pressure cycling technology (PCT) are carried out in the same processing container (PCT Shredder PULSE Tube). This method of extraction is safe, convenient, and efficient. Furthermore, the extracted DNA was far less sheared as compared to DNA extracted by bead beating. In addition to releasing longer DNA from plants, *The PCT Shredder* alone or in combination with the PCT SPS has proven to effectively release DNA, RNA, and proteins from fungi and *C. elegans*. *The PCT Shredder* is also an effective tool for pre-processing tissues such as cardiac muscle, liver, and lung. *The PCT Shredder* is powerful enough to release organelles and yet still gentle enough to preserve the intact structure of mitochondria released from tissues.

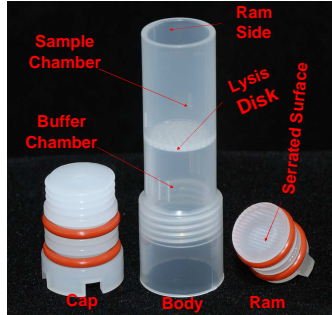
The PCT Shredder Driver & Holder



THE PCT SHREDDER

The PCT Shredder is a gentle, small, portable mechanical homogenization system for use with tough, fibrous, and other difficult-to-disrupt tissues and organisms. *The PCT Shredder* is used to rapidly homogenize a biological sample directly in the PCT Shredder PULSE Tube. This pre-processing method increases sample surface area and, when followed by PCT extraction (not always required), permits better access of high pressure extraction fluid to the sample, resulting in improved sample lysis and extraction of the desired analytes (Reference 1).

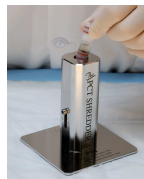
The PCT Shredder PULSE Tube



THE PCT SHREDDER PULSE TUBE

PCT Shredder PULSE Tubes are used to disrupt tough structures such as fibrous muscle tissue, plant cell walls, insect exoskeletons, or the cuticle of nematodes, to rapidly achieve excellent yields of high quality nucleic acids, proteins, lipids, and small molecules. The combination of *The PCT Shredder* and PCT Shredder PULSE Tubes may allow for the use of less aggressive lysis reagents due to the improved efficiency of sample extraction during pre-processing, whether followed by PCT or in some cases another sample preparation method.

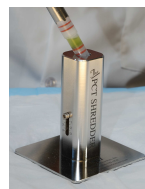
Placing the PULSE Tube in the Holder



Shredding the Sample with The PCT Shredder Driver



Leaf Sample Processed with The PCT Shredder



MATERIALS AND METHODS

The PCT Shredder may be used with different methods to optimize the yield of nucleic acids, proteins, lipids, and small molecules depending on the tissue type to be processed. Detailed processing methods are found in the *The PCT Shredder* Manual and other manuals and publications in which *The PCT Shredder* is described.

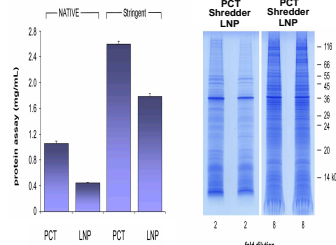
METHOD 1: The "FAST" or "Frozen Abrasive Shredder Technique" uses Silicon Carbide (SiC) and snap freezing, followed by shredding. This method is recommended for small organisms, such as *C. elegans* (Reference 2 and 3).

METHOD 2: Shredding with Silicon Carbide (SiC) Abrasive. SiC may be used with *The PCT Shredder* without flash freezing the sample. This method is recommended for tough animal and plant tissue.

METHOD 3: No Abrasive/Shredder Only. Softer tissue, such as liver, brain, and leaves, may be processed without SiC using *The PCT Shredder* alone.

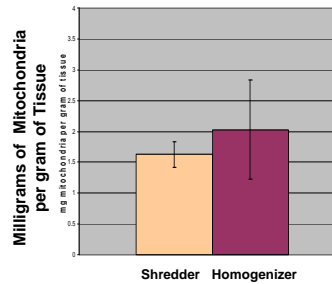
RESULTS

Protein Extracted from *C. elegans*



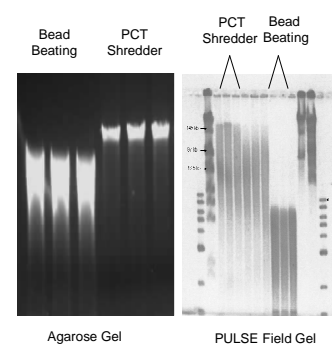
Extraction of Protein from *C. elegans* Using *The PCT Shredder* and PBI's ProteoSolve-CE Native and Stringent Kits ("FAST" Method) Results in Greater Yield of Protein than Extraction with Mortar and Pestle with Liquid Nitrogen (LNP). Reference 4.

Mitochondria Extracted from Rat Skeletal Muscle



Extraction of intact mitochondria from tissue is simplified by *The PCT Shredder* with comparable yields and greater reproducibility obtained compared to the labor intensive glass/Teflon homogenizer.

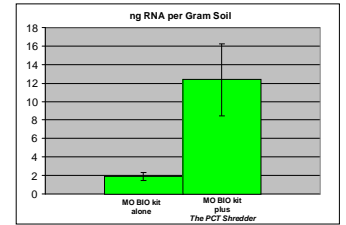
DNA Extracted from Spinach Leaf



DNA Extracted from Spinach Using *The PCT Shredder* Results in Much Longer and More Intact DNA Than Bead Beating While Providing Comparable Yields. Reference 5.

RNA Extracted from Soil

ng RNA/gm Soil MO BIO Protocol Alone	ng RNA/gm Soil MO BIO Kit Plus The PCT Shredder
1.6	14.5
1.9	14.5
1.7	6.5
2.5	14.0



Approximately 4-9 fold more RNA was extracted from Newfoundland soil using a MO BIO RNA PowerSoil™ Total RNA Isolation Kit* with the addition of a "shredding" step using *The PCT Shredder*™ from PBI than the MO BIO protocol alone (used as directed by the manufacturer).

FEATURES AND BENEFITS OF THE PCT SHREDDER

- Gentle, mechanical homogenization system
 - Small
 - Portable
 - Affordable
- Safely and rapidly breaks apart tough, fibrous, and other difficult samples, such as:
 - Plant and animal tissue
 - Arthropod exoskeletons
 - Cuticle of nematodes
- Increase yields of high quality nucleic acids, proteins, lipids, and small molecules
- Single-use processing containers
 - Inexpensive
 - Self-contained
 - No sample transfer required
- Excellent for collection, storage, transport, and processing of samples
- Use less aggressive buffers & reagents

REFERENCES

- The PCT Shredder* Manual: PBI
- ProteoSolve-CE Native Manual: PBI
- ProteoSolve-CE Stringent Manual: PBI
- Gabrielle E. Giese et al., 2009. "Phosphopeptide isolation from *Caenorhabditis elegans* using the ProteoSOLVE, PCT, and PhosphoScan Technologies" International HUPO 2009.
- Application Note: "Improved DNA Recovery from Spinach Leaves Using *The PCT Shredder*™ and Pressure Cycling Technology (PCT)": PBI