Gentle, Rapid, and Safe Disruption of Tissues and Organisms for the Extraction of DNA, RNA, Protein, Mitochondria, Lipid, and Small Molecules

The PCT Shredder or The SHREDDER SG3

Barocycler®, PULSE® Tubes, The PCT Shredder™, The SHREDDER SG3™, PCT Shredder PULSE Tubes™ and Shredder Tubes™ are trademarks of Pressure BioSciences, Inc.
PBI Shredder Systems are low shear mechanical devices for gentle, rapid, and safe disruption of tissues and organisms. Both The PCT Shredder and The SHREDDER SG3 can provide effective extraction of DNA, RNA, proteins, mitochondria, lipids and small molecules from tissues and organisms, when used with Shredder Tubes and various buffers.

Both systems use a variety of Shredder Tubes to directly and rapidly grind solid samples providing easy handling and reducing sample contamination. And, although not required for all extractions, additional extraction efficiency can be achieved when this process is combined with pressure cycling technology (PCT).

PBI SHREDDER SYSTEMS

Features and Benefits of The PCT Shredder
- Portable
- Sturdy, Stainless Steel Construction
- Cordless
- Compact and Lightweight driver

The PCT Shredder

Features and Benefits of The SHREDDER SG3
- Long Lasting Lithium Rechargeable Batteries
- Heavy Duty Robust Driver
- Three Position Lever for Setting Reproducible Force

The SHREDDER SG3
Variations and Specifications of Shredder Tubes

<table>
<thead>
<tr>
<th>Tube Type</th>
<th>Color</th>
<th>Metal Disk</th>
<th>PCT Compatible</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT Shredder PULSE Tubes FT500-S</td>
<td>White</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>PCT Shredder PULSE Tubes FT500-MS</td>
<td>White</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Shredder Tubes FT500-PS</td>
<td>Coral</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Shredder Tubes FT500-PMS</td>
<td>Coral</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Features & Benefits of the Shredder Tube:

- A Variety of Single-Use Processing Containers
- Standard Tubes for Ambient Pressure Processing
- Specialized Tubes for Ultra-High Pressure Processing
- Metal Inserts for the Toughest of Samples
- Excellent for Collection, Storage, Transport & Processing
- Closed Containers to Help Ensure Safety Throughout the Entire Sample Preparation Process
EXTRACTION OF DNA

DNA from Spinach Shown on a Pulsed Field Gel

DNA Extraction from spinach using the PBI Shredder System Results in Much Longer DNA than bead beating methods, while providing comparable yields.

DNA Extraction from Apple Seeds

Consistent high yields of DNA can be obtained from apple seeds using either PBI Shredder System.

Shredder Tubes (FT500-PMS) or PCT Shredder PULSE Tubes (FT500-MS) with metal inserts give more consistent and higher recovery of DNA from hard samples, such as apple seeds.

Agarose gel electrophoresis shows that PBI Shredder Systems yield higher molecular weight genomic DNA, while the bead beater causes significant shearing of DNA.
EXTRACTION OF RNA

High quality RNA suitable for the preparation of cDNA libraries can be obtained with *The PCT Shredder* or *The SHREDDER SG3*.

Shredder Tubes are compatible with a wide variety of sample preparation kits and reagents including commercially available chaotropes for the extraction of nucleic acids.

RNA from Rat Skeletal Muscle

![RNA Yield Graph](Image)

**EXTRACTION OF PROTEIN**

*Protein from the Nematode C. elegans*

Extraction of protein from *C. elegans* using *The PCT Shredder* and ProteoSolve-CE Native or ProteoSolve-CE Stringent Kits from PBI results in greater yield of protein than extraction with mortar and pestle with liquid nitrogen (LNP).

PBI’s ProteoSolve-CE Native Kit was developed for the efficient recovery of native proteins and protein complexes under minimally denaturing conditions in which native conformation and activity are preserved.
Protein from Rice Grains

Although PBI Shredder Systems are comparable in yield to mortar and pestle methods, they are far less labor intensive than manual techniques.

With PBI Shredder Systems there is no need to transfer the ground material for further processing.

Since Shredder Tubes are single-use disposables, cleaning between extractions is no longer necessary.

Protein from Rat Skeletal Muscle

Consistent high yields of protein can be obtained from muscle tissue using PBI Shredder Systems.

Protein yield data demonstrate that Dounce homogenization is less consistent (large error bar), while the Shredder samples are grouped much more tightly (small error bar), indicating greater reproducibility.

Note that better results may be obtained for some sample types using PCT Shredder PULSE Tubes (FT500-S) or Shredder Tubes (FT500-PS) without metal inserts. This may be due to the sticky nature of some samples, which can clog the smaller pores of the metal insert.
Protein from porcine cartilage (~30 mg per sample) extracted in PBI ProteoSolve-IEF Buffer.

Tissue disruption was performed with *The SHREDDER SG3* in PCT Shredder PULSE Tubes (FT500-MS) for 40 seconds at lever setting #2 or #3.

Comparison samples were disrupted using a commercially available homogenizer for 1 minute.

The negative control was generated by soaking a piece of cartilage in PBI ProteoSolve-IEF Buffer without mechanical disruption.
PROTEIN EXTRACTION WITHOUT LIQUID NITROGEN

TUMOR PROTEIN: PBI SHREDDER VS. CRYOGENIC GRINDING

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Target Protein</th>
<th>Cryogenic Grinding Yield</th>
<th>PCT Shredder Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metastatic Ovarian Tumor to The Omentum</td>
<td>FAS Ligand (a Single Transmembrane Segment Protein)</td>
<td>3±0.5 pg/mg Tissue</td>
<td>4.0±0.05 pg/mg Tissue</td>
</tr>
<tr>
<td>Fresh Frozen Prostate Cancer Tissue</td>
<td>Prostate-specific Membrane Antigen (a Single Transmembrane Segment 100K Protein)</td>
<td>~80% Recovery</td>
<td>~80% Recovery</td>
</tr>
<tr>
<td>Metastatic Ovarian Tumor to The Omentum</td>
<td>AKT1 (a Soluble Protein)</td>
<td>0.24 ng/mg Tissue</td>
<td>0.35 ng/mg Tissue</td>
</tr>
</tbody>
</table>

The PBI Shredder System can effectively replace liquid nitrogen grinding in some applications.

Cryogenic grinding (mortar and pestle with liquid nitrogen) followed by extraction with PBI’s ProteoSolve-TD2 Kit and pressure cycling technology (PCT) yielded less protein than disruption by The PBI Shredder System followed by extraction with PBI’s ProteoSolve-TD2 Kit and PCT.

ProteoSolve-TD2 Buffer in combination with PCT facilitates recovery of intact membrane proteins from solid tumors. ProteoSolve-TD2 Buffer contains no organic solvents and is directly compatible with most subsequent proteomic analyses (2-D gels, SDS PAGE, liquid chromatography, antibody assays, and mass spectrometry).

ProteoSolve-TD2 Buffer also recovers soluble cytosolic proteins to provide a more complete representation of the cellular proteome than can be achieved by other methods.
Yield and quality of mitochondria prepared from rat skeletal muscle with a PBI Shredder System compared to a Teflon/glass homogenizer are similar, but with far greater ease-of-use, lower cost and safety with the PBI system.

PBI offers specialized kits for the extraction of mitochondria from various tissues.

Mitochondria prepared from freshly harvested tissue using the PBI MITOCHONDRIA ISOLATION KIT: RAT MUSCLE are intact, normal and functional, as assessed by western blotting, electron microscopy, Ca^{2+} uptake and respiration assays.
PREPARING TICKS FOR DETECTION OF TICK-BORNE PATHOGENS

PBI Shredder vs. Mortar and Pestle

DNA Yield (μg DNA per mg Tick Mass)

Preparation of ticks for the detection of tick-borne pathogens is often done with a razor blade and mortar/pestle. This method is both hazardous and labor intensive.

 Recovering the processed tick from the mortar requires time and patience.

PBI Shredder Systems eliminate the labor and hazard of mortar and pestle preparation.

<table>
<thead>
<tr>
<th></th>
<th>Large ticks</th>
<th>Small ticks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shredder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/P</td>
<td></td>
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</table>
DETECTION OF THE CAUSITIVE AGENT OF LYME DISEASE (*Borrelia burgdorferi*)

Photographic Insert: A classic presentation of a tick bite showing a characteristic “bulls eye” rash, termed Erythema migrans indicating the potential for Lyme disease.

Plot: Real-time PCR detection of *Borrelia burgdorferi*, the causative agent of Lyme disease extracted from the tick that inflicted the bite in the photograph.

Data indicate that this tick harbored *B. burgdorferi* equivalent to approximately 830,000 infectious organisms.
SHREDDING TREE BARK

Better shredding of bark is obtained using PCT Shredder PULSE Tubes (FT500-MS) or Shredder Tubes (FT500-PMS) with metal inserts.

PBI Shredder Systems are ideal for processing small samples of bark (<25mg).

The difference in the color of the extract between plum and pine bark is due to tannin content, not the effectiveness of shredding.
ADVANTAGES OF THE PBI SHREDDER SYSTEMS

Simple, Easy-To-Use
Inexpensive Hardware and Disposable Sample Containers
Compatible with Routinely Used Lab Buffers and Other Reagents
Single-Use, Closed, Versatile Processing Container
  • Self-Contained
  • No Sample Transfer Required
  • Reduces the Likelihood of Cross Contamination
  • Reduces the Chance of Sample Exposure to the User
  • Reduces the Chance of User Exposure to Potentially Hazardous Samples
  • Excellent for Collection, Storage, Transport, and Processing
  • Offers Full “Chain-of-Custody” Tracking from Collection to Test
  • Available with Metal Inserts for Use with Hard Samples Such as Seeds
  • Reduces Sample-to-Sample Variability

Safe – No Exposed Sharp Surfaces
Portable – Excellent for Field Use