ORDERING INFORMATION

To order *The* SHREDDER SG3 or Shredder Tubes and other accessories, please contact your local Pressure BioSciences Sales Representative.

Or, please visit: http://www.pressurebiosciences.com



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Discovery Starts With Sample Preparation

The SHREDDER SG3

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

User Manual



For Research Use Only

Not for Use in Diagnostic Procedures

Barocycler®, PULSE®Tubes, *The PCT Shredder™*, *The* SHREDDER SG3™ PCT Shredder PULSE Tubes™ and Shredder Tubes™ are trademarks of Pressure BioSciences. Inc.

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New Procedures are added periodically to the PBI Website.

APPLICATIONS NOTES AND PrEPS

http://www.pressurebiosciences.com/pub/application-notes-preparations.html

- Isolation of Functional Mitochondria from Rat Skeletal Muscle Using The PCT Shredder with an Optional Pressure Cycling Technology (PCT) Processing Step
- 2. Isolation of Mitochondria from Rat Lung Using The PCT Shredder and Pressure Cycling Technology (PCT)
- 3. Proteolysis (Proteinase K)-PrEP: Efficient and Rapid Extraction of Genomic DNA from Skeletal Muscle using The PCT Shredder and Pressureenhanced Proteolysis with Proteinase K
- 4. Increased Protein Yields from Coniferous Plants Using The PCT Shredder and Pressure Cycling Technology (PCT)
- 5. Improved Protein Recovery from Muscle Tissue Using The PCT Shredder and Pressure Cycling Technology (PCT):Efficient Single Tube Sample Disruption and Extraction
- 6. Improved DNA Recovery from Spinach Leaves Using The PCT Shredder and Pressure Cycling Technology (PCT)

PROTOCOLS for The SHREDDER SG3

The following Table lists some applications and general conditions for *The* SHREDDER SG3. For complete protocols, consult the Application Notes and PrEPs under Shredder Applications.

Some General Conditions for The SHREDDER SG3

| Application | Shredder Tube | Shred Time | Lever Position |
|---|----------------------------------|------------------|-------------------|
| DNA Extraction from Spinach Leaves | FT 500-S (coral or white) | 20-30 seconds | 2 |
| DNA Extraction from Whole Apple Seeds | FT 500-MS (coral or white) | 30-40 seconds | 2 |
| Protein Extraction from Skin or Mus- cle Tissue | FT 500-S (coral or white) | 20-30 seconds | 2 |
| RNA from Muscle Tissue | FT 500-S (coral or white) | 20-30 seconds | 2 |
| RNA from Rodent Lung Tissue | FT 500-MS (coral or white) | 20-30 seconds | 2 |
| Protein from Whole Rice Grains | FT 500-MS (coral or white) | 30-60 seconds | 2 |
| Protein from Porcine Cartilage | FT 500-MS (coral or white) | 40-60 seconds | 3 |

SAFETY INFORMATION

Standard laboratory safety practices should be employed when working with chemicals to minimize potential exposure to hazardous materials.

PRODUCT USE LIMITATIONS

The SHREDDER SG3TM is intended for **Research Purposes Only**. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

PBI warrants to the original purchaser only that all *The* SHREDDER SG3 parts will be free from defects in material and workmanship for a period of ninety (90) days from the date of purchase. Seller's sole obligation and your exclusive remedy under this limited warranty and, to the extent permitted by law, any warranty or condition implied by law, shall be the repair or replacement of parts, without charge, which are defective in material or workmanship and which have not been misused, carelessly handled, or repaired by persons other than PBI or authorized service representative.

If *The* SHREDDER SG3 does not meet your expectations, please contact our Technical Service Department. PBI reserves the right to change, alter, or modify any product to enhance its performance and design.

TECHNICAL ASSISTANCE

At PBI we pride ourselves on the quality and availability of our technical support. Our Technical Service Department is staffed by experienced scientists with

extensive practical and theoretical expertise in sample preparation, proteomics, molecular biology and the use of PBI products. If you have any questions or experience any difficulties regarding *The* SHREDDER SG3 or other PBI products or services, please do not hesitate to contact a Technical Service Representative at:

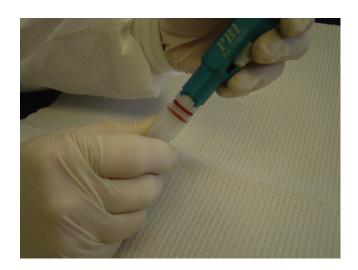
technicalsupport@pressurebiosciences.com

INTRODUCTION

The SHREDDER SG3 is a low shear mechanical homogenization system for use with tough, fibrous and other difficult-to-disrupt tissues and organisms. The SHREDDER SG3 uses a variety of Shredder Tubes to directly and rapidly grind a biological sample providing easy handling and reducing sample contamination. This low shear mechanical disruption process, when combined with selected buffers, can provide effective extraction of proteins, DNA, RNA, lipids and small molecules from tissues and organisms. Additional extraction efficiency can be achieved when this process is combined with pressure cycling technology (PCT), resulting in further improvement of sample lysis and extraction of the desired analytes.

The SHREDDER SG3 features a three position force setting lever (FSL). Otherwise it is similar in function to PBI's *The PCT Shredder*. The FSL enables the operator to select and apply <u>reproducible</u> force to the sample during the shredding process. Further, the FSL eliminates the need for the operator to exert force for long periods when processing one or more samples.

4



To recover any residual sample, gently push the Shredder Ram with the Shredder Tube Tool until the Ram presses on the Lysis Disk. Aspirate the remaining sample. In some cases, it may be necessary to remove particulates from the homogenate by brief centrifugation prior to downstream processing.

Shred as long as necessary until all, or most, of the sample has passed through the Lysis Disk to the upper chamber of the Shredder Tube. It may be necessary to shred for several seconds using a lower lever setting before completing shredding on a higher setting.

Note: If PCT treatment will follow shredding, the final volume of sample and reagents in the tube must be adjusted to 1.4 \pm 0.1 mL prior to placing the sample into the Barocycler.

Heat Generation While Shredding

Many samples, including most kinds of tissue samples, require only 20-30 seconds of shredding. While all shredding will produce some heat due to friction, this short processing time will not significantly heat the sample.

However, harder samples like tree bark and seeds may need to be shredded for a longer time to achieve maximum homogenization. In these cases more heat will be generated. To minimize heat generation during longer shredding protocols, the stainless steel SHREDDER Base can be pre-chilled by placing it into a refrigerator or a -20°C freezer.

Note: Shredding non-stop for 2 minutes is not recommended as it may result in decreased protein recovery due to heat-induced protein aggregation.

Removing the Processed Homogenate

To remove the processed homogenate, use the Shredder Tube Tool to open the Shredder Tube by removing the Shredder Cap. Transfer the sample to a micro-centrifuge tube or to another appropriate container

HARDWARE SUPPLIED with The SHREDDER SG3

| | Kit Components | Part Numbers |
|----------|---|--------------|
| 1A 1B | One Heavy Duty High Torque SG3 Driver with a Convertible Handle | 22-3022 |
| 2 | One SG3 Base with a 3 position Force Setting Lever (FSL) | 22-3021 |
| 3 | One Battery Charger | 22-3023-001 |
| 4 | Two Lithium Ion Batteries | 22-3023-002 |
| 5 | One Shredder Tube Tool | 110639 |
| 6 | One Assortment Package of Shredder Tubes | FT500-SV |

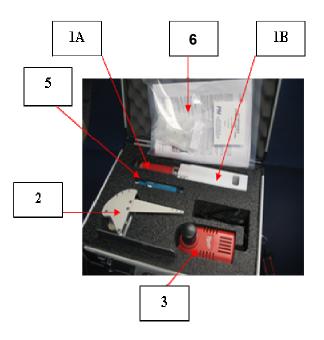


Figure 1: *The* SHREDDER SG3 Kit Components Shown in Case. Batteries Not Shown.



Figure 2: *The* SHREDDER SG3 *Assembled* for Shredding

VARIATIONS AND SPECIFICATIONS OF SHREDDER TUBES

| Tube Type | Color | Metal Disk Insert | PCT Compatible |
|--|-------|-------------------------|-------------------|
| PCT Shredder PULSE Tubes FT500-S | White | No | Yes |
| PCT Shredder PULSE Tubes FT500-MS | White | Yes | Yes |
| Shredder Tubes FT500-PS | Coral | No | No |
| Shredder Tubes FT500-PMS | Coral | Yes | No |

PBI offers a variety of Shredder Tubes for various applications. Only white Shredder Tubes are approved for use with high pressure sample preparation.

While pressing down on *The* SHREDDER SG3 Driver with one hand, set the lever into the appropriate position (1-Low, 2-Med, or 3-High) for the sample.



For hard samples, such as seeds or rice, forcing the lever down to position 3 when shredding a sample larger than 25 mg or samples of soft tissue greater than 100 mg may damage the Lysis Disk. It is best to shred briefly at position 2 until most of the sample is shredded. Then proceed to position 3 to complete the shredding.



Cap the Shredder Tube using a Shredder Screw Cap with the Shredder Tube Tool.



Place the filled Shredder Tube into the SHREDDER Base, Ram side down, and twist to set the Ram into the holder in the SHREDDER Base. When the Tube is seated securely, place the SG3 Driver onto the Cap, and briefly turn the driver on in order to seat the Driver bit into the crenellations of the Cap.



SHREDDER APPLICATIONS REQUIRING PRESSURE CYCLING TECHNOLOGY (PCT)

PCT is not require for all shredder preparations. However, some preparations are enhanced when shredding is followed by PCT.

Please refer to Page 15 or the PBI website under Application Notes and Preparations (PrEPs) for specific examples of PCT-enhanced extraction.

http://www.pressurebiosciences.com/pub/application-notes-preparations.html

To obtain more information about a Barocycler for high pressure extractions, please refer to the PBI website (www.pressurebiosciences.com) or ask your Sales Representative how PCT may enhance your particular application.



Figure 3. A PBI Barocycler NEP2320

PREPARING The SHREDDER SG3

First, fully charge one of the two lithium ion batteries. Insert the charged battery into the SG3 Driver. These batteries can be recharged at any time and do not have to be fully discharged before recharging.

HOW TO USE The SHREDDER SG3

Place approximately 10 to 300 mg of sample into the Ram side of an appropriate Shredder Tube.



Using the Shredder Tube Tool, insert a serrated Shredder Ram with a twisting motion so that the sample is pressed between the serrated surface and the Lysis Disk.



Determine the method, Shredder Tube type or reagent kit appropriate for your specific application by consulting the Table on Page 14 of this manual, or by reviewing Application Notes and PrEPs on the PBI website.

The Shredder SG3 and Shredder Tubes are compatible with a wide variety of sample preparation kits and reagents such as Trizol for RNA extraction as well as kits sold by PBI for specialized sample preparation applications.

http://www.pressurebiosciences.com/pub/application-notes-preparations.html

Add 200-700 μ L of appropriate extraction reagent to the Cap side of the tube. The total volume of sample and buffer during shredding should not exceed ~0.7-0.8 mL (This prevents buffer from being forced into the threads of the Cap where it might be lost during uncapping).

