APPLICATION NOTE #201508

Protein Extraction from Soft Tissues using the Bead Ruptor Elite

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Introduction

Proteomic profiling attempts to elucidate not only protein repertoire, but the structure and function of proteins. Post genome sequencing, scientists are now focusing on studying what proteins are decoded from the genome. The first step in many proteomic studies is the extraction and isolation of proteins from the sample of interest. The success of a protein assay is often dependent on quality and method of this first step. A common method for extraction of proteins from animal tissues is mechanical homogenization. While many tissue types can be robust and require significant mechanical force to dissociate them and access their cellular components, tissues like brain and liver are quite soft and require a gentler homogenization approach in order to ensure adequate sample extraction without sacrificing the molecular integrity of the proteins of interest. This is especially important for proteomic studies aiming to analyze tertiary structure, enzyme activity or post-translational modifications.

The Bead Ruptor Elite is a bead mill homogenizer capable of processing up to 24 tissue samples per cycle and has been used in a number of studies for the isolation of proteins from soft tissues. 1,2,3 Bead mills function by the high speed shaking of a sample in a sealed tube in the presence of beads. The beads impact the sample with sufficient force to dissociate both the tissues and the cells.

In this study, we demonstrate protein extraction from soft tissues through bead milling using the Bead Ruptor Elite. Efficiency and analyte integrity were evaluated.

Materials & Methods

Equipment

- **Bead Ruptor Elite** (Cat #19-040E)
- **Bead Ruptor, 2 mL Tube Carriage Kit**(Cat #19-010-310)
- **2 mL Soft Tissue Homogenizing Mix** (Cat #19-627)

Procedure

Rattus norvegicus brain, liver, lung and spleen samples were placed into 2 mL non-reinforced tubes containing 0.57g of 1.4 mm ceramic beads (Cat #19-627). 500 µL of Tris-buffered saline was added to each tube and the samples were homogenized on the Bead Ruptor Elite for one cycle at the settings described in Table 1. Processing speed and duration were adjusted based on the toughness and size of the tissue samples (Table 1). Homogenized mixtures were immediately centrifuged at 8,000 x g for 10 minutes. 1.5 µl of the supernatant was used to determine protein concentrations at 280 nm on a Nanodrop spectrophotometer (Thermo-Fisher).

2 µl of the protein extract was mixed with 5 µl Laemmli sample buffer(BioRad), heated at 95°C for 5 minutes, then 4 µL of each sample and 5 µL of protein ladder were loaded and separated by SDS-PAGE for 30 mins at 200V on a 4-20% Tris Glycine SDS polyacrylamide gel (BioRad). Proteins were stained with Coomassie, destained and visualized on a GelDoc EZ system (BioRad).

Sample Size (mg)	Speed (m/s)	Time
Brain (70 mg)	4.85 m/s	20 sec
Liver (70 mg)	5.30 m/s	20 sec
Lung (68 mg)	5.50 m/s	20 sec
Spleen (58 mg)	4.85 m/s	20 sec

Figure 1: Sample size and Bead Ruptor Settings

Results

In this study, the Bead Ruptor Elite's ability to process a variety of soft tissue in order to extract proteins was evaluated. High concentrations of protein from all soft tissue samples were detected post homogenization. Concentrations were determined by spectrophotometry as shown in Table 2. The highest concentration was from spleen at an average of 57.468 mg/mL and the lowest concentration was from brain at 10.687 mg/mL. SDS-PAGE analysis indicating a broad protein repertoire was obtained from each sample with abundant bands observed across the entire molecular weight range.

Sample Type	Concentration (mg/mL)
Brain	10.687
Liver	24.438
Lung	32.666
Spleen	57.468

Figure 1: Protein concentration of samples

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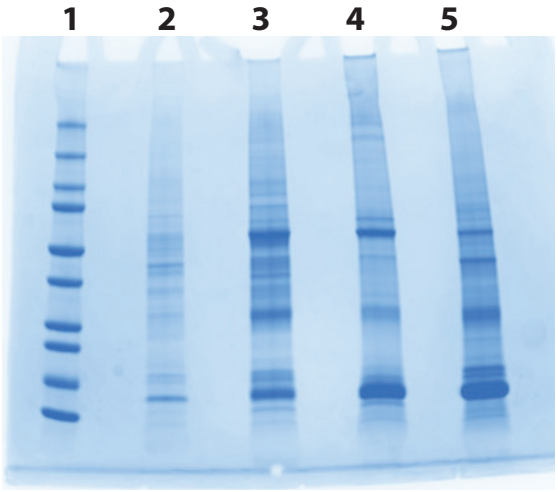

Lane 1: 10kD Ladder
Lane 2: Brain
Lane 3: Liver
Lane 4: Lung
Lane 5: Spleen

Figure 1: SDS-PAGE Analysis of soft tissue samples

Conclusion

The Bead Ruptor Elite is capable of homogenizing soft tissue samples in less than 30 seconds to obtain adequate protein yields as a starting point for many proteomic applications. Due to the nature of different tissue types, different processing speeds and times were required to complete sample disocation. The Bead Ruptor Elite was demonstrated to provide rapid protein extraction of up to 24 samples.

References

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Bead Ruptor Elite: (Cat #19-040E)

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