

Protein Extraction from Soft Tissues using the Fisher Scientific Bead Mill 24

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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 Mill 24 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 Mill 24. Efficiency and analyte integrity were evaluated.

Materials & Methods

Equipment

- **Bead Mill 24** (Cat No. 15340163)
- **2 mL Soft Tissue Homogenizing Mix** (Cat No. 15340153)

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 No. 15340153). 500 µL of Tris-buffered saline was added to each tube and the samples were homogenized on the Bead Mill 24 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

Table 1 Sample size and Bead Mill 24 Settings

Results

In this study, the Bead Mill 24'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

Table 2 Protein concentration of samples

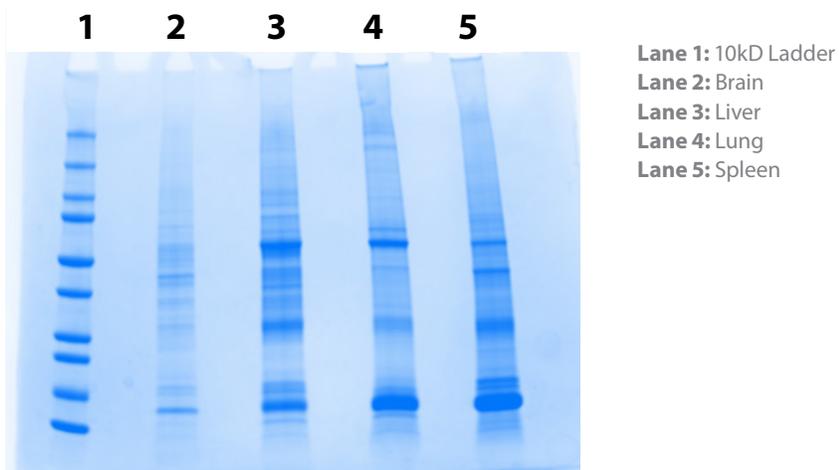


Figure 1: SDS-PAGE Analysis of soft tissue samples

Conclusion

The Bead Mill 24 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 dislocation. The Bead Mill 24 was demonstrated to provide rapid protein extraction of up to 24 samples.

References

1. Shishido et al. "Bioavailability and Efficacy of a Gap Junction Enhancer (PQ7) in a Mouse Mammary Tumor Model." *Plos One* 8.6 (2013). Plos One. Web. 30 Sept. 2015. <<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0067174>>.
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Bead Mill 24: (Cat #15340163)