APPLICATION NOTE #20145

# Protein Extraction from Hard Tissue Samples using the Bead Mill 24

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The Bead Mill 24 enables rapid and efficient tissue homogenization for a variety of samples types. The Bead Mill 24 is capable of homogenizing up to twenty-four samples in tube sizes ranging from 0.5 mL to 50 mL using optimized bead matrices to facilitate sample disruption. Hard samples such as muscle, skin and bone represent a significant challenge for extraction of target analytes such as proteins and nucleic acids.

Proteins are an important target in scientific research. They serve as biomarkers for disease, targets for therapeutic purposes, and provide essential physiological functions in living organisms. Extraction and recovery of a sample's proteome is often the first step in proteomic research. However, tissue composition variability creates a significant barrier for developing a universal extraction method. Although soft tissue types are easier to disrupt, hard tissues such as bone, skin, and muscles are difficult to disrupt quickly without degrading the analyte of interest.

Traditional disruption methods can include incubation in the presence of harsh chemicals or pulverization under cryogenic conditions.[1,2] For optimal protein recovery, an efficient and reproducible method is necessary that is effective across a broad range of sample types.

Herein, we demonstrate protein extraction from tough tissues via bead milling in the Bead Mill 24.

## Materials and Methods

All rat tissues were obtained from Bioreclamation, Inc . Skin, tail, tongue and back muscle samples were manually sectioned and placed into 2 mL reinforced polypropylene tubes filled with five 2.8 mm ceramic beads (Cat #15340154). 1 mL of Tris-HCl, pH 7.6 was added to each tube and the samples were homogenized on the Bead Mill as described in Table 1

Homogenized mixtures were immediately centrifuged at 8,000 g for 10 minutes. 1.2 µl of the supernatant was used to determine protein concentrations at 280 nm on a Nanodrop spectrophotometer (Table 1).

10 µl of the protein extract was mixed with 5 µl Laemmli sample buffer, heated at 90°C for 10 mins, then proteins were separated by electrophoresis for 30 mins at 200V on a 4-20% Tris Glycine SDS polyacrylamide gel. Proteins were stained with coomassie and visualized on a GelDoc EZ system (BioRad).

**Table 1 Sample Size and Bead Mill 24 Settings**

| Sample Type (mg) | Speed (m/s) | Cycle Time (s) |
|---|---|---|
| Skin (90) | 7.3 | 45 s |
| Tail (70) | 6 | 45 s |
| Tongue (90) | 7.45 | 3 x 30 s |
| Back Muscle (45) | 6 | 45 s |
| Bone (250) | 6.95 | 8 x 45 s (45 s dwell) |

## Results

Protein extraction from tough tissues such as skin and muscle represent a significant challenge and bottleneck for proteomic research. Traditional approaches such as chemical lysis or cyromilling are not reproducible and are difficult to scale. Bead milling, as supported in the Bead Mill 24, increases protein extraction throughput and enables homogenization of tough biological tissues.

In this study rat skin, tail, tongue, muscle and bone tissues were homogenized in the Bead Mill 24 and protein yields were quantified by spectrophotometry. Table 2 indicates that bead milling resulted in high protein yields from all tissue types including bone.

Protein extracts were further separated and visualized by SDS-PAGE (Figure 1). Skin and tail produced very similar protein repertoires due to the fact that both tissue types are similar in that the tail is largely composed of skin tissues. Tongue tissue, while requiring the most amount of force to generate a full homogenate, generated a significant protein yield and the largest molecular weight variation among its visualized proteins (Table 2, Figure 1).

Protein extraction from bone samples represent a unique challenge and are typically achieved through a chemical decalcification process. In this study full homogenization was achieved directly through bead beating without the need the tedious chemical lysis process. Both high protein yields and a broad protein repertoire was achieved through the bead milling process (Table 2, Figure 2).

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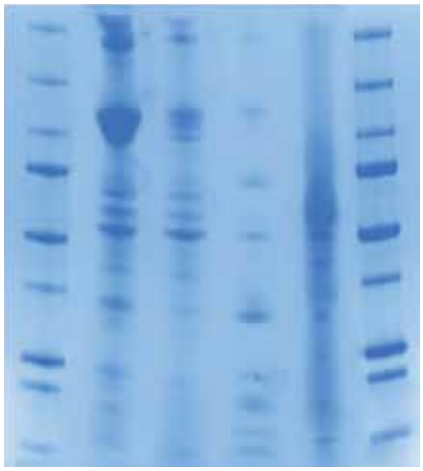

**Figure 2 Protein Gel Electrophoresis on Tough Tissues.** Polyacrylamide SDS PAGE electrophoresis of protein extractions. Lane 1: protein ladder. Lane 2: skin. Lane 3: tail. Lane 4: back muscle. Lane 5: tongue.

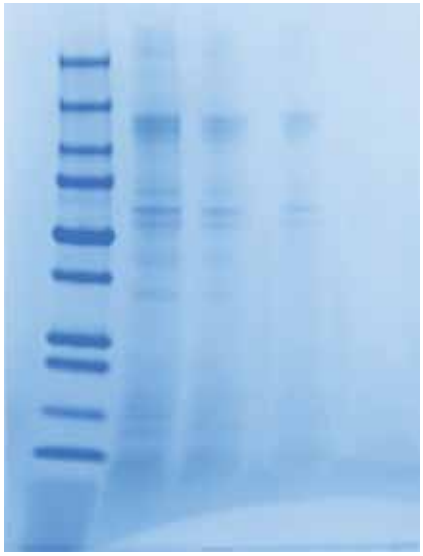

**Figure 3 Protein Gel Electrophoresis on Bone Protein Extracts.** Polyacrylamide SDS PAGE electrophoresis of bone protein extractions. Lane 1: protein ladder. Lane 2: bone protein extract. Lane 3: 2X dilution of bone protein extract. Lane 4: 3X dilution of bone protein extract.

**Table 2 Sample Type and Protein Yield**

| Sample Type | Protein Yield (µg/µL) |
|---|---|
| Skin | 2.374 |
| Tail | 18.204 |
| Tongue | 1.286 |
| Back Muscle | 14.395 |
| Bone | 0.722 |

## Conclusion

The Bead Mill 24 facilitates rapid protein extraction from tough tissues and bone. Bead milling eliminates the need for strong acids or expensive buffer systems and provides for reproducible homogenization.

## References


1. Jiang X et al. Method Development of Efficient Protein Extraction in Bone Tissue for Proteome Analysis. *Journal of Proteome Research* **2007** 6: 2287-2294.

2. Zakharchenko O et al. Optimized Protocol for Protein Extraction from the Breast Tissue that is Compatible with Two-Dimensional Gel Electrophoresis. *Breast Cancer: Basic and Clinical Research* **2011** 5: 37-42.


*Fisher Scientific Bead Mill 24*

## Part Numbers Referenced

**Bead Mill 24 Motor Unit:** 15340163

**2 mL Pre-Filled Hard Tissue Homogenizing Mix:**

15340157

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