APPLICATION NOTE

Bead mill “Beadless” protein extraction from soft tissues.

The Omni Bead Ruptor Elite™ bead mill homogenizer vigorously shakes tubes filled with bead material to disrupt samples such as soft tissues. The impact between the beads and the sample reduces sample particle size and creates a homogenous mixture. While bead milling is an efficient method of homogenization, for some applications, the presence of the bead media within the sample can be a drawback. This is certainly the case for small samples that are heat sensitive. Heat produced through the bead milling process results from the kinetic energy generated from the beads impacting the sample and tube walls. Heat generation can be problematic when extracting volatile or heat sensitive samples such as proteins or RNA.

Herein we evaluate the feasibility of a bead mill based “beadless” homogenization for protein extraction from soft tissues. Protein extraction efficiency is analyzed following a homogenization procedure in which tissues are homogenized on the Omni Bead Ruptor Elite bead mill homogenizer in tubes without beads present.

Materials and methods

Equipment

- Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E)
- 2 mL Tube with Screw Caps (Cat # 19-647)
- Soft Tissue Homogenizing Mix 1.4 mm Ceramic (2 mL) (Cat # 19-627)
- Omni Bead Ruptor Elite 2 mL Tube Carriage (Cat # 19-373)

For research use only. Not for use in diagnostic procedures.

Procedure

Two Sprague-Dawley rat brain tissue sections (Bioreclamation Inc.) ranging in mass from 21 to 26 mg were placed into two 2 mL polypropylene screw cap tubes (Cat# 19-647) (Table 1). To two additional tubes, brain tissue sections were added to 2 mL tubes pre-filled with 1.4 mm ceramic bead media. To all samples, 500 µL of Tris-HCL (pH 7.6) was added to each sample tube. Tissue samples were homogenized on the Omni Bead Ruptor Elite bead mill homogenizer (Cat#19-042E) fitted with a 2 mL tube carriage (Cat#19-373) at 5 m/s for 17 seconds. The tissues were centrifuged at 11,000 g for 3 minutes. The supernatant was removed and 1 µL was used to determine the protein concentration at absorbance 280 nm on the Nanodrop spectrophotometer. Proteins were visualized on a 4-20 % Tris-Glycine SDS polyacrlyiminde gel, by mixing 5 µL of sample to 5 µL of laemmli sample buffer. The mixture was heated at 95 °C for 5 minutes and then separated via SDS-PAGE for 30 minutes at 200 V then stained in coomassie blue for 1 hr. The gel was visualized on a GelDoc EZ imaging system after destaining overnight in DD H2O.

Results

Duplicate rat brain samples were homogenized with and without 1.4 mm ceramic bead media in the Omni Bead Ruptor Elite bead mill homogenizer for 17 seconds. Visual inspection confirmed that in both cases full homogenization was achieved with no indication of solid tissue material present post homogenization. Protein yields were quantified by spectrophotometry and displayed in Table 1 and Figure 2. Detected protein concentrations were normalized by starting tissue mass to compare yields between bead and beadless processing. Protein yields were shown to not vary between the two approaches with the average concentration ranging from 1 to 1.6 mg/mL.

The protein profiles were further evaluated by SDS-PAGE to determine if the protein repertoire was affected by processing without the presence of bead media (Figure 1). SDS-PAGE analysis indicated that there was no discernible difference in protein banding between tissue samples extracted with and without beads.

Table 1. Starting tissue sample mass and normalized protein yields.

Sample type	Average protein yields (mg/mL)	Tissue mass (mg)	Normalized concentration
1.4 mm beads	1.353	23	1.235
1.4 mm beads	2.057	26	1.662
Beadless	1.073	21	1.073
Beadless	1.324	21	1.324

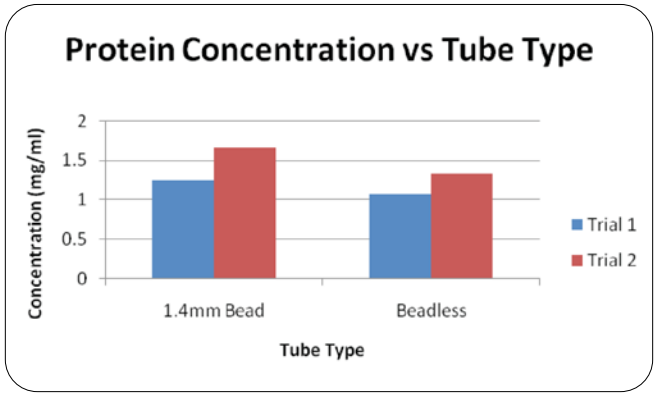

Figure 1. Normalized protein yields resulting from tissue extraction with and without beads.

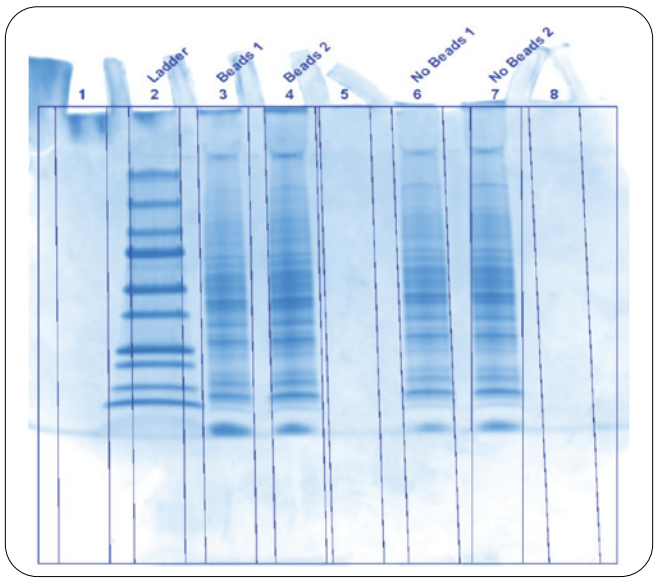

Figure 2. Rat brain protein extracts visualized by coomassie blue stained SDS-PAGE. Lane 2: Ladder. Lane 3: 1.4 mm beads. Lane 4: 1.4 mm beads. Lane 6: Beadless. Lane 7: Beadless.

Conclusions

The central dogma of bead milling is that beads must be present in the sample to produce a homogenous mixture. Here we evaluated if homogenization could be performed on the Omni Bead Ruptor Elite bead mill homogenizer using tubes containing sample, buffer and no beads. Protein extraction was successfully performed using the beadless approach with comparable yields and protein repertoires when compared to the traditional bead based approach.

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