APPLICATION NOTE

Rapid protein extraction from Tissues with the Omni Bead Ruptor 4 bead mill homogenizer.

The Omni Bead Ruptor 4 (BR 4) bead mill homogenizer takes up a small footprint on the bench while still retaining the homogenizing power provided with Revvity's bead ruptor family. The BR 4 is designed to efficiently lyse tissues for extraction of intracellular compounds, and is capable of homogenizing up to four 0.5 mL, 1.5 mL or 2 mL tubes or one 7 mL tube using bead media to assist in sample disruption. Screw cap or micro tubes containing bead media specific for the sample of interest are vigorously shaken to disrupt samples and release target analytes. Since the disruption occurs very quickly the molecular integrity of the target compound is maintained. This is particularly important for maintaining enzyme activity or for heat sensitive samples such as RNA.

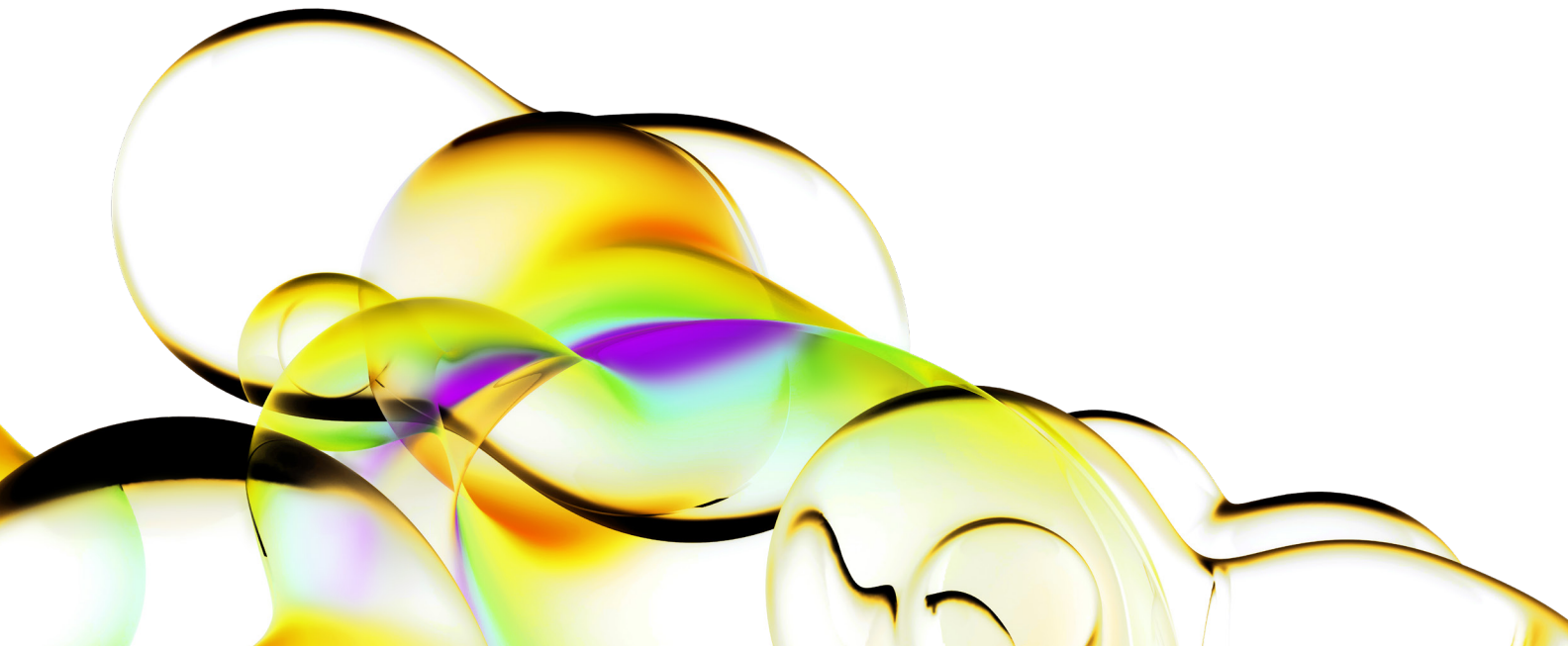
Here, we demonstrate protein extraction from various tissue types using the Omni Bead Ruptor 4. Both protein extraction efficiency and diversity of proteins extracted are evaluated.

Materials and methods

Equipment

- **Omni Bead Ruptor 4 bead mill homogenizer** (Cat # 25-010)
- **Hard tissue homogenizing mix 2.8 mm ceramic beads** (Cat # 19-628)

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



Sample preparation

Approximately 25-30 mg of Sprague-Dawley rat muscle, brain, liver and heart tissue samples (Bioreclamation Inc.) were homogenized in 750 µL of Tris-HCL (pH 7.6) in 2 mL polypropylene screw cap tubes pre-filled with 2.8 mm ceramic beads (Cat # 19-628) in duplicate. Brain, liver and muscle tissues were processed in the Omni Bead Ruptor 4 (Cat # 25-010) at a speed of 4 for one cycle of 45 seconds. Heart tissues were processed at speed 4 for two 45 second cycles with a 1 minute dwell between each cycle. The homogenates were centrifuged at 11,000 x g for 10 minutes. The supernatant was removed and 1 µL was analyzed on a Nanodrop spectrophotometer at absorbance 280 nm to determine protein concentrations. The protein concentrations were then normalized based on starting tissue mass.

Protein separation

Ten micrograms of each protein extract was added to Laemmli sample buffer (BioRad, Cat # 1610747) and heated at 95 °C for 5 minutes. Proteins were separated on a 4-20% Tris-Glycine SDS polyacrylamide gel (BioRad, Cat # 4568096) in a Mini Protean Tetra gel electrophoresis chamber at 200V for 45 minutes. Proteins were stained in coomassie blue for 1.5 hours then destained in water overnight. The gel was then visualized on a GelDoc EZ System (BioRad).

Results

After processing on the Omni Bead Ruptor 4 all tissue samples were homogenized to completion as confirmed by visual inspection. Protein concentrations were then quantified on the Nanodrop spectrophotometer and shown in Table 1 and Figure 2. Due to the variation in starting tissue mass, the protein concentrations were normalized to the lowest starting mass. The normalized protein concentrations are displayed in Table 1 and Figure 2. The highest protein yields were achieved from the rat brain and liver samples. Rat back muscle tissue yields were lowest at 0.55 and 0.79 mg/mL.

Table 1. Tissue sample mass and protein concentrations following homogenization on the Omni Bead Ruptor 4

Tissue type	Starting tissue mass (mg)	Measured protein concentration (mg/mL)	Normalized protein concentration (mg/mL)
Muscle sample 1	25	0.62	0.55
Muscle sample 2	28	1.01	0.79
Brain sample 1	29	2.88	2.18
Brain sample 2	28	3.35	2.63
Liver sample 1	30	1.97	1.44
Liver sample 2	22	2.17	2.17
Heart sample 1	26	1.31	1.11
Heart sample 2	29	1.88	1.43

Results (cont.)

Ten protein repertoires were visualized for each tissue by gel electrophoresis and staining (Figure 1). The high protein concentrations determined by spectrophotometry were further validated by the observance of abundant protein banding on the stained protein gel. Figure 1 shows that all tissue types produced strong protein bands with a broad molecular weight range.

Conclusion

The Omni Bead Ruptor 4 is capable of extracting proteins from various tissues effectively in a short amount of time. The protein extraction between all tissue types was completed successfully with high protein yields and proteins were observed with a broad molecular weight range through SDS-PAGE.

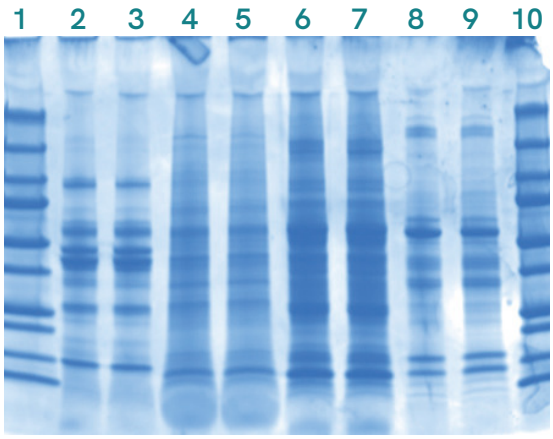


Figure 1: Rat tissue proteins visualized by SDS-PAGE.
Lane 1: Protein Ladder. Lane 2: Muscle 1 Lane 3: Muscle 2.
Lane 4: Brain 1. Lane 5: Brain 2. Lane 6: Liver 1. Lane 7: Liver 2.
Lane 8: Heart 1. Lane 9: Heart 2. Lane 10: Protein Ladder.

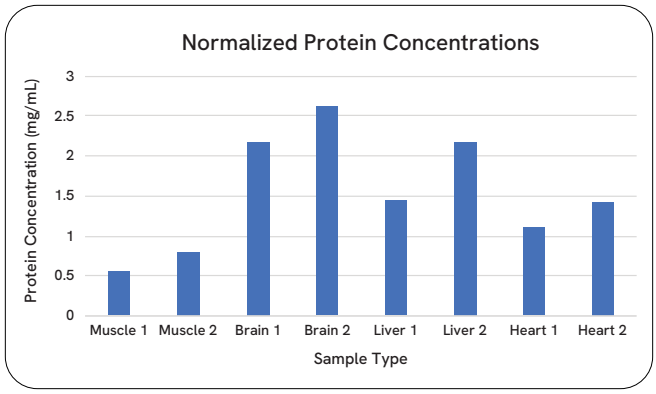


Figure 2: Normalized protein concentrations based on rat tissue type

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com