APPLICATION NOTE #20141

Evaluating the Reproducibility of Protein Extraction from Hard Tissues Using the Bead Ruptor 12 Homogenizer

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Homogenization is often the first step in a tissue proteome study. It has been shown that the accuracy of proteome studies are largely dependent on the sample preparation method utilized. The Omni International Bead Ruptor 12 offers a fast and reproducible solution for tissue homogenization resulting in high protein recovery.

Materials and Methods

Rat heart tissue samples were dissected into eight 50 mg samples, placed in Omni International 2ml reinforced homogenization tubes containing 0.49 g of 2.8 mm yttria stabilized zirconia oxide bead media (Cat#19-628), and diluted with 1 ml of 50 mM Tris-HCl, pH 7.6. Tissues were homogenized in the Bead Ruptor 12 on High for 60 sec. Insoluble material and beads were pelleted by centrifugation at 10,000 rpm for 10 min. Protein yields were determined by absorbance measurements at 280 nm on a Nanodrop spectrophotometer (Thermo Fisher). 10 µl of homogenate was mixed with 5 µL of Laemmli sample buffer and proteins were separated by SDS-PAGE on a 4-20% Tris-Glycine TGX gel (BioRad) at 200 V for 30 min. Proteins were stained with G 250 Coomassie BioSafe Stain (Bio-Rad) and visualized on a BioRad Gel-Doc EZ.

Results

The Omni Bead Ruptor 12 enables simultaneous homogenization of 12 X 2ml, 12 X 1.5ml, 12 X 0.5ml and 4 X 7ml samples. The ability to process all eight heart tissues in a single run ensured that each tissue was homogenized with the same force and for the same amount of time thus removing errors associated with manual homogenization. Here, complete homogenization was achieved in 60 seconds. This rapid homogenization is feasible in the Bead Ruptor 12 through an optimized tube carriage motion resulting in vertical bead movement maximizing bead impact forces. For tough samples such as heart tissue high impact forces are critical to ensure that samples are processed under non-denaturing conditions and without excessive heat generation, which can lead to analyte degradation. Protein quantification measurements indicated a reproducible protein yield of 7.2 +/- 0.71 mg from 50 mg of starting tissue mass with an average yield deviation of less than 10%. Analysis of the protein extracts by SDS-PAGE indicated a high degree of reproducibility in the overall protein repertoire (Figure 2). Similar banding and migration patterns were observed for each of the eight homogenates indicating that a reproducible extraction was achieved in both the total protein yield and individual protein constituents.

Conclusion

Quantitative protein measurements such as those employed in proteomic studies are greatly facilitated by reproducible sample preparation methodologies that produce high analyte yields. Homogenization of eight rat heart tissue samples was accomplished in 60 seconds using the Bead Ruptor 12. Spectrophotometric and electrophoresis analysis indicated a reproducibly high protein yield across all eight samples. The Bead Ruptor 12 is an ideal homogenization platform facilitating high throughput protein extraction.

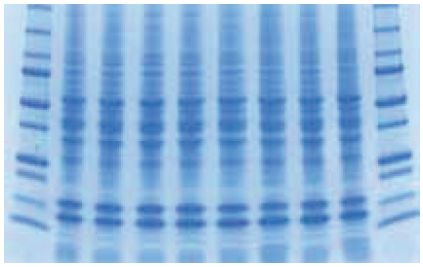

Figure 2. Omni Bead Ruptor 12 delivers reproducible results for high throughput sample homogenization of heart tissue.

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