APPLICATION NOTE

Total protein quantification from umbilical cord tissue using the Omni Bead Ruptor Elite bead mill homogenizer for sample preparation.

Summary

Processing and analysis of umbilical cord tissue has become a widespread method to analyze biomarkers that diffuse into tissue cells and may only be present in the blood for a short period of time[1]. Analysis of umbilical cord tissue, directly, by common methods like ELISA, Western blot and LC-MS/MS often gives researchers a more comprehensive picture to regulation of key indicators in gestational syndromes like pre-eclampsia, gestational diabetes[1].

Upstream of analysis methods is sample preparation, which is frequently accomplished manually, chemically or a combination of the two. The Omni Bead Ruptor Elite™ bead mill homogenizer is a quick and efficient way to overcome the harsh and time-consuming nature of alternative homogenization methods, while still producing a homogenate suitable for downstream analysis.

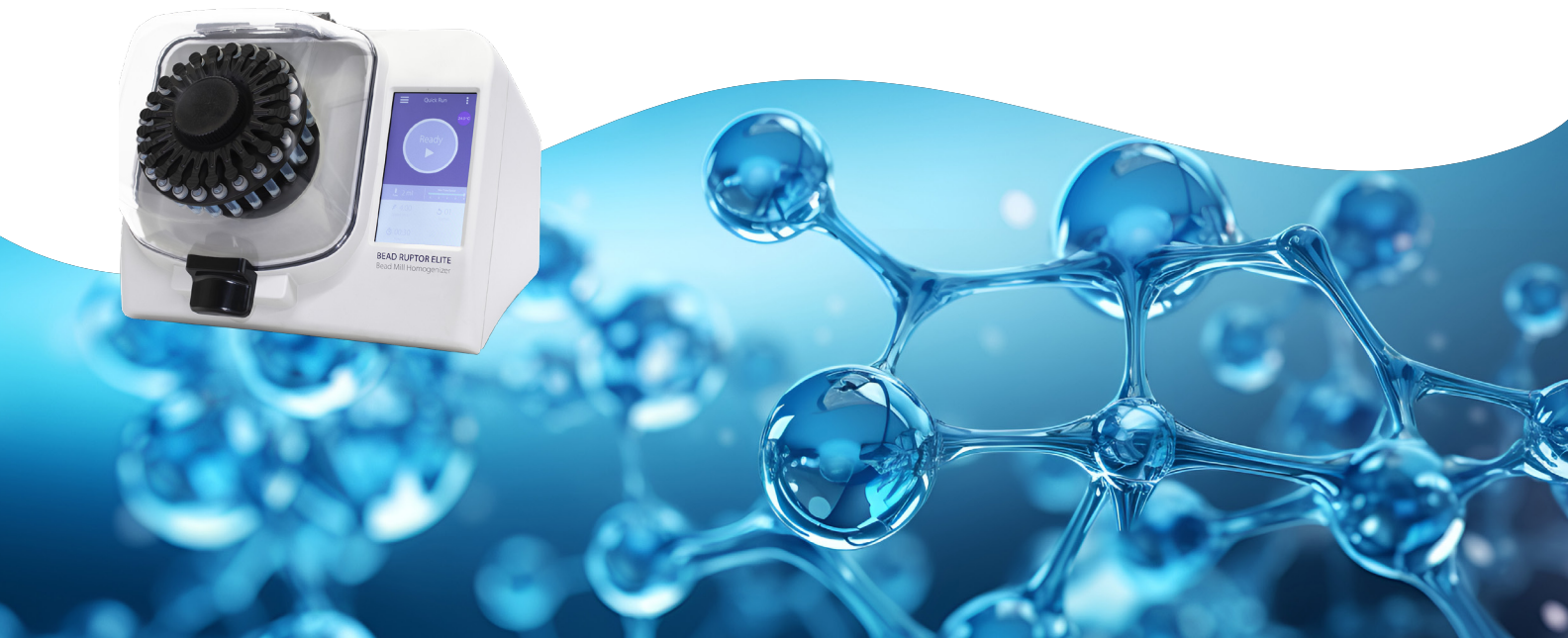
Herein, we outline sample preparation of fixed umbilical cord tissue on the Omni Bead Ruptor Elite for downstream total protein quantification.

Authors

Gabriella Ryan, M.S.
Rodney J Nash, Ph.D.

Revvity, Inc.

Omni Bead Ruptor Elite bead mill homogenizer



Materials and methods

Equipment

- Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E)
- Omni Bead Ruptor Elite 2 mL Tube Carriage (Cat # 19-373)
- Hard Tissue Homogenizing Mix 2.8 mm Ceramic (2 mL) (Cat # 19-628)

Procedure

Sample acquisition

De-identified paraformaldehyde-fixed human umbilical cord samples were obtained from Emory University Hospital, Tissue Procurement Lab under standard IRB protocols.

Total protein BCA assay

200 mg of umbilical cord was weighed out and transferred to a 2 mL Hard Tissue Homogenizing Mix Tube (Cat # 19-628) along with 600 µL of phosphate buffered saline (PBS) (Gibco, Cat # 20012027). Samples were weighed out with a tolerance ± 10 mg. Umbilical cord samples were processed on the Omni Bead Ruptor Elite bead mill homogenizer at 5 m/s for 2 cycles of 30 seconds (Table 1). Homogenate was then transferred to a microplate and prepared according to the instructions for 'Microplate Procedure' in the BCA Protein Assay Kit (Thermo Fisher Scientific, Cat # 23225). Working Reagent and Bovine Serum Albumin (BSA) standards A-G (Table 2) were also prepared according to dilution scheme provided in the kit instructions. Prepared microplate sample absorbance was measured at 562 nm, along with included BSA Standards. Blank 562 nm absorbances were used for further construction of standard curve and analysis of measured protein concentration.

Table 1: Umbilical cord sample homogenization summary.

Sample type	Sample weight (mg)	Speed (m/s)	Time (sec)	Cycles	Dwell time (sec)
Umbilical cord	200	5	30	2	10

Results

Homogenization of umbilical cord on the Omni Bead Ruptor Elite yielded a complete homogenate after 1 minute of processing time (Figure 1). An average protein concentration of 1237 µg/mL was recovered from homogenized umbilical cord tissue (Table 3). Protein concentration from the sample was determined based on the equation generated from the standard curve.

Figure 1: Pre- and post-processing images of 200 mg umbilical cord.

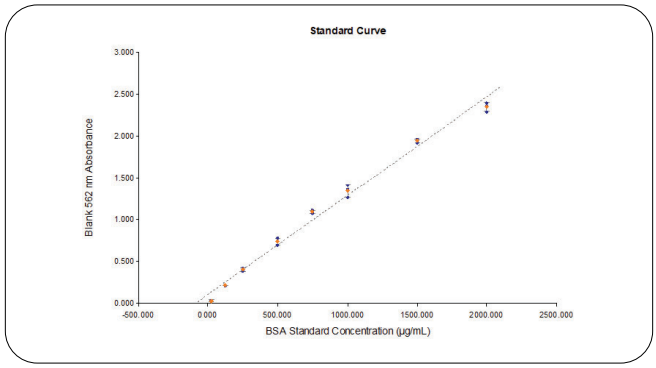


Figure 2: Standard curve generated using BSA standards A-G from the BCA Protein Assay Kit. Equation generated from standard curve: y=mx + b, where y is defined as the Blank 562 nm measurement, m is defined as 0.00118, b is defined as 0.104 and x is protein concentration (µg/mL).

Table 2: Average BSA Standard Protein Concentration between prepared triplicate standards.

Standard	Known concentration (µg/mL)	Average measured concentration (µg/mL)
A	2000	1904
B	1500	1562
C	1000	1051
D	750	837
E	500	536
F	250	235
G	125	92
PBS	0	0

Table 3: Calculated protein concentrations from umbilical cord tissue.

Sample	Calculated protein concentration (µg/mL)
1	1055
2	1282
3	1372

Conclusions

The Omni Bead Ruptor Elite along with 2 mL Hard Tissue Homogenizing Mix provides a sample preparation solution to tough samples, like umbilical cord. By producing a homogenate suitable for downstream analysis, high concentrations of total protein were recovered, indicating that homogenized samples may be plugged in to more analytical assays like ELISA, Western blot, and LC-MS/MS.

Refrences

1. Knight SJ, Smith AD, Wright TE, Collier AC. Detection of opioids in umbilical cord lysates: an antibody-based rapid screening approach. Toxicol Mech Methods. 2019;29(1):35-42. doi:10.1080/15376516.2018.1506850

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