APPLICATION NOTE

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

Authors

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

Revvity, Inc.

Summary

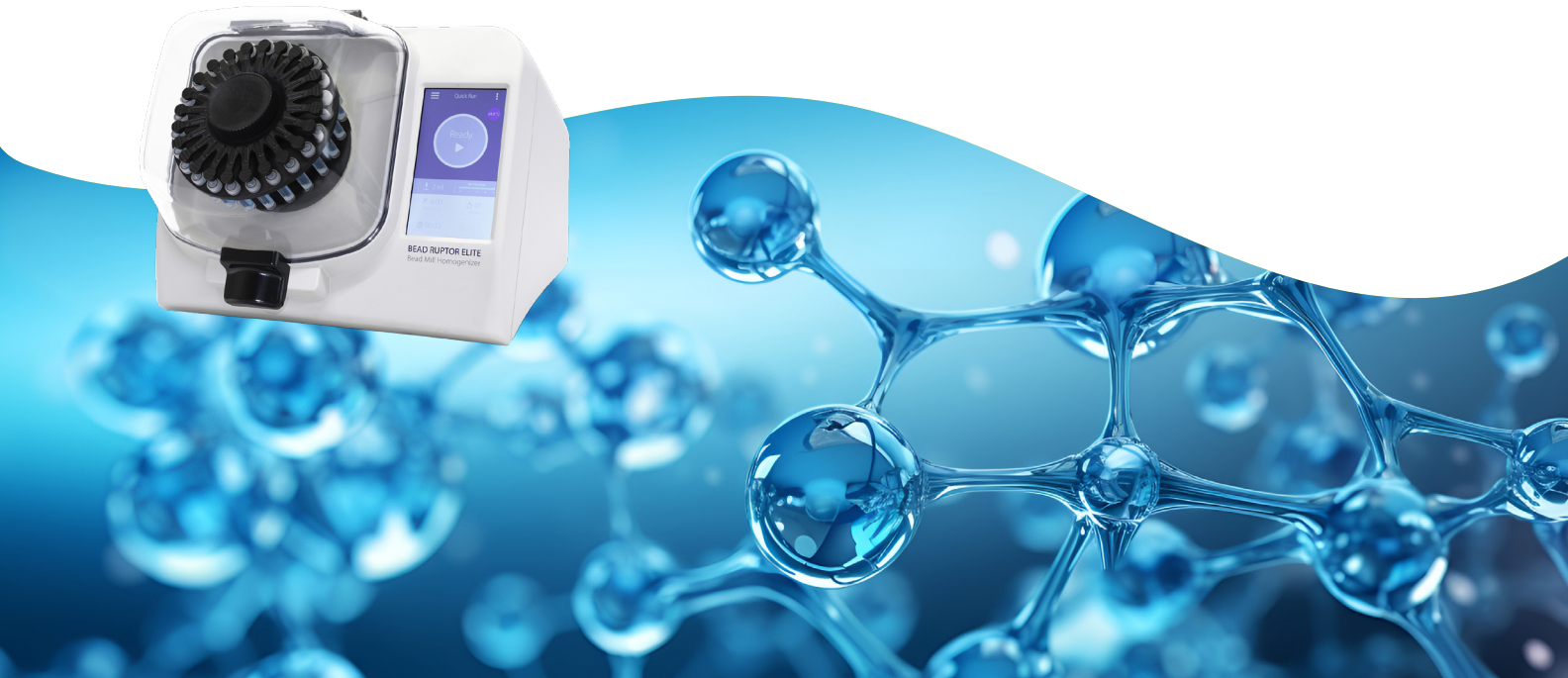
The impact of proteomics research on placenta tissue has allowed scientists to discover patterns in key biomarkers related to both placental and gestational syndromes and pathologies[1]. Specifically, at the bench, uncovering changes in biomarkers related to preeclampsia[2], gestational diabetes[3], and TORCH[4] (Toxoplasmosis, "Others", Rubella, Cytomegalovirus, Herpes simplex virus) syndrome, to name a few, are propelling researchers and clinicians forward in being able to diagnose and treat these conditions during pregnancy.

Upstream of protein-based assays like Western blot, ELISA, LC-MS/MS is a sample preparation step involving homogenization of fixed placenta tissue, which lyses cells and releases analytes of interest. Standard homogenization methods involving harsh detergents and enzymes can be time consuming and ineffective procedures for tough samples like placenta. Bead mill homogenization on the Omni Bead Ruptor Elite™ bead mill homogenizer is a quick and effective alternative to standard chemical-based sample preparation methodologies, while still producing a homogenate suitable for downstream analysis.

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

Omni Bead Ruptor Elite bead mill homogenizer

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



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)
- 2 mL Hard Tissue Homogenizing Mix (Cat # 19-628)

Procedure

Sample acquisition

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

Total protein BCA assay

200 mg of placenta tissue was weighed out and transferred to a 2 mL Hard Tissue Homogenizing Mix Tube (Cat No. 19-628) along with 600 µL of phosphate buffered saline (PBS) (Gibco, Cat # 20012027). Samples were weighed out with a tolerance ± 10 mg. Placenta 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: Placenta sample homogenization summary.

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

Results

A complete homogenate was obtained after 1 minute of processing time (Figure 1). Downstream BCA Total Protein quantification yielded an average protein concentration of 1064 µg/mL (Table 3). Protein concentration was determined based on the equation generated from the standard curve, using BSA standards for measurement of absorbance at 562 nm.

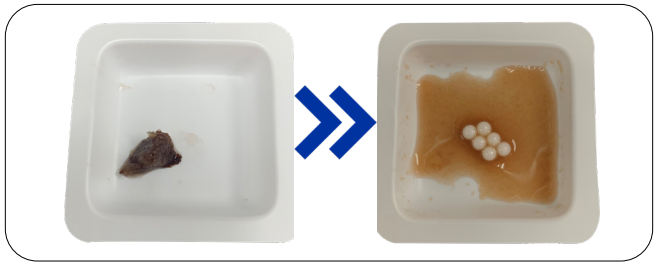


Figure 1: Pre- and post-processing images of 200 mg placenta tissue.

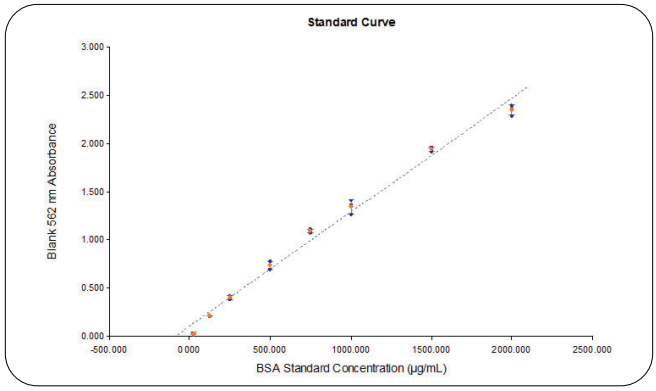


Figure 2: Standard curve generated using BSA standards A-G from the BCA Protein Assay Kit. Equation generated from standard curve: y=mx + b, where y is defined as the Blank 562 nm measurement, m is defined as 0.00118, b is defined as 0.104 and x is protein concentration (µg/mL).

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 homogenized placenta tissue.

Sample	Calculated protein concentration (µg/mL)
1	938
2	1129
3	1123

Conclusions

The Omni Bead Ruptor Elite bead mill homogenizer along with 2 mL Hard Tissue Homogenizing Mix offers scientists a sample preparation solution for placenta tissues yielding a complete homogenate compatible for downstream total protein quantification. As seen in the above experiments, high yield protein concentration recovered from homogenized placenta is a suitability indicator for further proteomics assays like ELISA, LC-MS/MS, and Western blot.

References

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4. Macedo-da-Silva J, Marinho CRF, Palmisano G, Rosa-Fernandes L. Lights and Shadows of TORCH Infection Proteomics. Genes (Basel). 2020;11(8):894. Published 2020 Aug 5. doi:10.3390/genes11080894

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