

# Total Protein Quantification from Murine Teeth Using the Bead Ruptor 96 for Sample Preparation

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Bead Ruptor 96™  
Bead Mill Homogenizer

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## Summary

Murine teeth are used as a model for study of topics relating to gene ontology, identification, and enamel analysis, allowing for downstream proteomic assays (Jágr, 2014). Prior to conducting specific assays relating to protein, the sample must be homogenized to lyse cells and release analytes of interest. Traditional lysis methods for tough sample types frequently involve mortar and pestle, liquid nitrogen, and enzymatic cocktails, which can be laborious inefficient procedures.

The Bead Ruptor 96 and the durable 2 mL Stainless Steel Grinding/Lysing tube provide scientists with an efficient front-end sample preparation solution to homogenize teeth samples, yielding homogenates that are suitable for downstream total protein quantification and setting the stage for in-depth protein analysis.

Herein, we assess the Bead Ruptor 96 and 2 mL Stainless Steel Grinding/Lysing tube in homogenization of murine teeth for incorporation into downstream BCA Total Protein assay.

## Procedure

### Total Protein BCA Assay

250 mg of teeth were weighed out and transferred to 2 mL Stainless Steel Grinding/Lysing Tubes (OMNI Cat No. 19-6001). Samples were weighed out with a tolerance  $\pm 10$  mg. Samples were processed on the Bead Ruptor 96 at 28 Hz for 30 seconds (Table 1). After processing, 500  $\mu$ l of phosphate buffered saline (PBS) (Gibco, Cat No. 20012027) was added to the powdered homogenate and the tube was mixed using the Vortex Mixer 24 (OMNI Cat No. 28-1001) at maximum speed for 30 seconds. Homogenate was then transferred to a microplate and prepared according to the instructions for 'Microplate Procedure' in the BCA Protein Assay Kit (Thermo Scientific, Cat No. 23225).



Working Reagent (WR) and Bovine Serum Albumin (BSA) standards A-G 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.

Sample Type	Sample Weight (mg)	Speed (Hz)	Time (sec)	Cycles
Murine Teeth	250 mg	28 Hz	30 sec	1

Table 1. Sample Homogenization Summary

Standard	Known Concentration (µg/mL)	Average Measured Concentration (µg/mL)
A	2000	1993
B	1500	1441
C	1000	1075
D	750	770
E	500	538
F	250	253
G	125	98
PBS	0	0

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

## Results

After homogenization of 250 mg of murine teeth on the Bead Ruptor 96, the resulting homogenate yielded an average calculated protein concentration of 438 µg/mL. Protein concentration was determined based upon the equation generated from the standard curve, using BSA standards for measurement of absorbance at 562 nm.

Sample	Calculated Protein Concentration t(µg/mL)
1	472
2	435
3	409

Table 3. Calculated Protein Concentrations. Protein concentration was calculated from the equation generated from the standard curve.

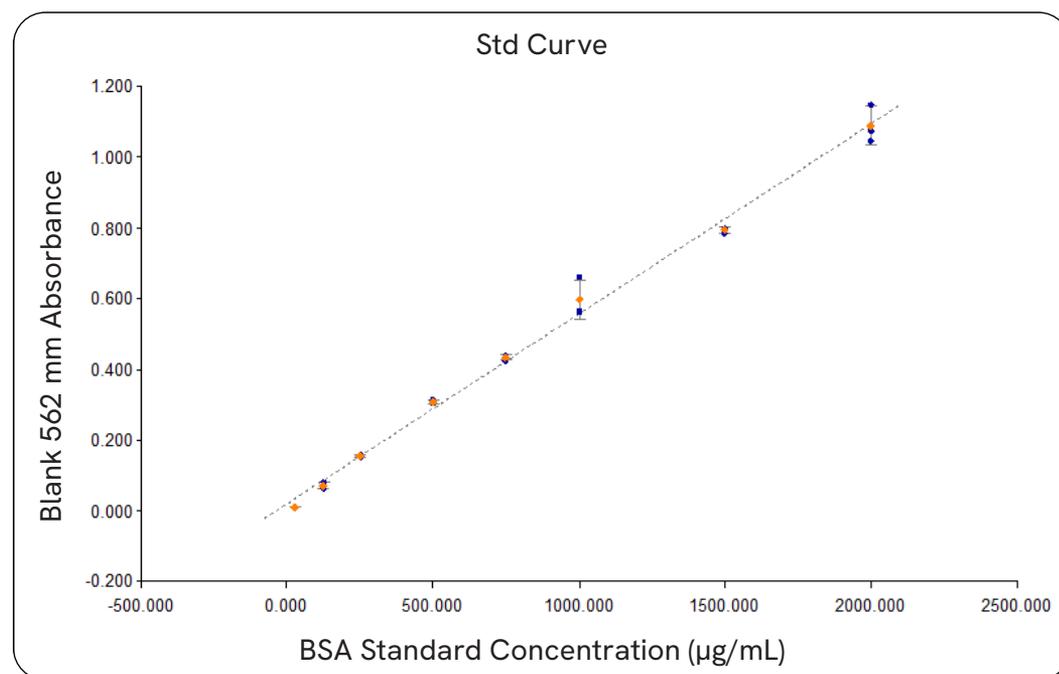


Figure 1. Standard curve generated using BSA standards A-G from the BCA Protein Assay Kit. Equation generated from standard curve:  $y = mx + b$ , where  $y$  is defined as the Blank 562 nm measurement,  $m$  is defined as 0.000537,  $b$  is defined as 0.0196 and  $x$  is protein concentration (µg/mL).

## Conclusions

The Bead Ruptor 96, along with the 2 mL Stainless Steel Grinding/Lysing tubes, offers a robust sample preparation solution to process tough samples, like murine teeth. The resulting homogenate is suitable for downstream total protein recovery and can be used as an indication for further proteomics assays like gene ontology analysis and ELISA.

Although the data was obtained using a 2 mL stainless steel tube, it should be noted that the experiment can be scaled up using the OMNI Stainless Steel Milling Jars (OMNI Cat No. 27-004; 27-006), if the initial sample size exceeds that of the 2 mL stainless steel tube.

## References

1. Jágr, M., Eckhardt, A., Pataridis, S., Broukal, Z., Dušková, J., & Mikšík, I. (2014). Proteomics of human teeth and saliva. *Physiological research*, 63 (Suppl 1), S141-S154. <https://doi.org/10.33549/physiolres.932702>



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