



Dec 10, 2020

Bicinchoninic acid Acid Protein Concentration measurement

Jcprice¹¹Brigham Young University*In Development*

This protocol is published without a DOI.

Chemistry 586 Advanced Biochemical Methods

Jcprice

PROTOCOL CITATION

Jcprice 2020. Bicinchoninic acid Acid Protein Concentration measurement . **protocols.io**
<https://protocols.io/view/bicinchoninic-acid-acid-protein-concentration-meas-bqkvmuw6>

LICENSE

This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Dec 10, 2020

LAST MODIFIED

Dec 10, 2020

PROTOCOL INTEGER ID

45429

MATERIALS TEXT

CuSO₄ and Bicinchoninic Acid Solution for the "Working Reagent",
PBS as diluent,
Clear plastic flat bottom 96 well plate
Experimental samples with protein concentration somewhere between 2 mg/mL and 0.02 mg/mL
37 Deg celsius shaking incubator
UV-Vis plate reader

DISCLAIMER:

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

1 Thaw the samples on ice.

2 Prepare standards using Pierce Protein Standard-2mg/ml, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, 0.062

mg/mL, 0.031 mg/mL, 0.016 mg/mL, 0 mg/mL

- 3 Organize your well plate and draw a map on the lid. Group your standards and each dilution of your experimental standards to simplify the analysis.
- 4 Make dilutions depending on the amount of protein expected. For BioID samples, measure lysate and 1/5 dilution in lysis buffer, by loading 25ul of each sample dilution into 2 wells. Label the lid to indicate where each sample and dilution is found
- 5 Load 25 ul of each standard into 2 wells. Label the lid again.
- 6 Prepare the "Working Reagent" immediately before use by mixing CuSO₄ and the Bicinchonic Acid Solution in a 1:50 ratio (need 200ul per sample or standard).
- 7 Add 200ul of Working Reagent into each well. Use a multichannel pipet to quickly add the reagent to all wells containing sample or standard.
- 8 Begin the lag-time, a 15 min incubation of the microplate at 37°C with shaking
- 9 Read the absorbance at 562nm on plate reader (ProteinQuant_BCA)
C:/Program Files/Molecular Devices.../Protein Quant/BCA.ppr (SoftMax Pro 5.2 rev C) Analysis
- 10 Graph the standards with the absorbance on the x-axis and the concentration on the y-axis. Standards with absorbance greater than 1.5 should not be used in the standard curve as the light signal is extremely low and overly noisy.
- 11 Fit standards data to a line using the trendline in excel or similar program. The min and max absorbance represent the bounds for signal allowable for your unknown standards. Although a polynomial fit could be used to fit the non-linear portions of a standard curve, this 9-point curve doesn't adequately constrain higher order polynomials so it is better to reduce the range of the curve and focus on the linear region.
- 12 Using the parameters from the linear regression calculate a concentration for each unknown (x should be absorbance). If multiple dilutions of the unknown are within the bounds of the standard curve these readings should be averaged together unless there is evidence that the measurement was done incorrectly i.e. replicates are extremely different.