

Lab 9: Quantitation of Proteins Using the BCA Protein Assay

Relevant Readings:

- Appendix A, page 141 of this manual
- Appendix B, page 147 of this manual
- Appendix C, page 149 of this manual
- Appendix E, page 159 of this manual
- Appendix F, page 163 of this manual

Exercise Objectives — Upon successful completion of this exercise you should be able to:

1. Give 2 examples of protein assays.
2. Name the protein assay used in this course.
3. State under what circumstances the BCA assay cannot be used.
4. State the number of steps involved in the BCA assay.
5. Describe or illustrate the reaction that occurs in the first step of the BCA assay.
6. List the factors that determine the rate of color development in the BCA assay.
7. Give two reasons to use the BCA assay.
8. Explain why BCA assay is not a true end-point method.
9. Explain the relationship between color intensity and concentration in the BCA assay.
10. Calculate the amount of protein in each fraction.
11. Calculate the total percent of protein in each fraction.

1. Introduction

There are a number of different protein assays commercially available. During a protein assay a compound is added to the sample that will change color in the presence of protein, the higher the concentration of protein in the sample, the greater the color change. The two types of protein assay most frequently used in labs are ones based on protein binding Coomassie blue dye (Bradford assay) and assays based on protein chelation with copper (Lowry and BCA assays). Choice of protein assay depends on ease of use and the compatibility of the assay with compounds present in the protein extract. While all commercially available protein assays are relatively quick and easy to perform in the lab, you will be using the BCA protein assay because it is not affected by the presence of detergents in protein samples. The BCA assay cannot be used if reducing agents (ex. mercaptoethanol) are present. Because your samples do not contain reducing agents this is not a problem.

The BCA assay consists of two steps. In the first step the protein reacts with Cu^{2+} under alkaline pH to produce Cu^{+} , producing a blue-colored product. The amount of product

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Reva Arnez Curry



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