Methods in Molecular Biology

Volume 1.

PROTEINS

Edited by

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Preface

In recent years there has been a tremendous increase in our understanding of the functioning of the cell at the molecular level. This has been achieved in the main by the invention and development of new methodology, particularly in that area generally referred to as "genetic engineering". While this revolution has been taking place in the field of nucleic acids research, the protein chemist has at the same time developed fresh methodology to keep pace with the requirements of present day molecular biology. Today's molecular biologist can no longer be content with being an expert in one particular area alone. He/she needs to be equally competent in the laboratory at handling DNA, RNA, and proteins, moving from one area to another as required by the problem he/she is trying to solve. Although many of the new techniques in molecular biology are relatively easy to master, it is often difficult for a researcher to obtain all the relevant information necessary for setting up and successfully applying a new technique. Information is of course available in the research literature, but this often lacks the depth of description that the new user requires. This requirement for in-depth practical details has become apparent by the considerable demand for places on our Molecular Biology Workshops held at Hatfield each summer. This book is therefore an attempt to provide detailed protocols for many of the basic techniques necessary for working with DNA, RNA, and proteins. This volume gives practical procedures for a wide range of protein techniques. A companion volume (Volume 2) provides coverage for nucleic acids techniques. Each method is described by an author who has regularly used the technique in his or her own laboratory. Not all the techniques described necessarily represent the state-of-the-art. They are, however, dependable methods that achieve the desired result.

Each chapter starts with a description of the basic theory behind the method being described. However, the main aim of this book is to describe the practical steps necessary for carrying out the method successfully. The Methods section therefore contains a detailed step-by-step description of a protocol that will result in the successful execution of the method. The Notes section complements the Methods section by indicating any major problems or faults that can occur with the technique, and any possible modifications or alterations.

This book should be particularly useful to those with no previous experience of a technique, and, as such, should appeal to undergraduates (especially project students), postgraduates, and research workers who wish to try a technique for the first time.

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Contents

Chapter 1

The Lowry Method for Protein Quantitation

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Introduction

The most accurate method of determining protein concentration is probably acid hydrolysis followed by amino acid analysis. Most other methods are sensitive to the amino acid composition of the protein and absolute concentrations cannot be obtained. The procedure of Lowry et al. (*1*) is no exception, but its sensitivity is moderately constant from protein to protein, and it has been so widely used that Lowry protein estimations are a completely acceptable alternative to a rigorous absolute determination in almost all circumstances where protein mixtures or crude extracts are involved.

Materials

1. *Complex-forming reagent*: prepare immediately before use by mixing the following 3 stock solutions A, B, and C in the proportion 100:1:1, respectively.

Solution A: 2% (w/v) Na_2CO_3 in distilled water
Solution B: 1% (w/v) $CuSO_4 \cdot 5H_2O$ in distilled water
Solution C: 2% (w/v) sodium potassium tartrate in distilled water

2. *2N NaOH*
3. *Folin reagent* (commercially available): Use at *1N* concentration.
4. *Standards*: Use a stock solution of standard protein (e.g., bovine serum albumin fraction V) containing 4 mg/mL protein in distilled water stored frozen at − 20°C. Prepare standards by diluting the stock solution with distilled water as follows:

Stock solution	µL	0	1.25	2.50	6.25	12.5	25.0	62.5	125	250
Water	µL	500	499	498	494	488	475	438	375	250
Protein concentration	µg/mL	0	10	20	50	100	200	500	1000	2000

Method

1. To 0.1 mL of sample or standard, add 0.1 mL of *2N* NaOH. Hydrolyze at 100°C for 10 min in a heating block or a boiling water bath.
2. Cool the hydrolyzate to room temperature and add 1 mL of freshly mixed complex-forming reagent. Let the solution stand at room temperature for 10 min.
3. Add 0.1 mL of Folin reagent, using a Vortex mixer, and let the mixture stand at room temperature for 30–60 min (do not exceed 60 min).
4. Read the absorbance at 750 nm if the protein concentration was below 500 µg/mL or at 550 nm if the protein concentration was between 100 and 2000 µg/mL.
5. Plot a standard curve of absorbance as a function of initial protein concentration and use it to determine the unknown protein concentrations.

Notes

1. If the sample is available as a precipitate, then dissolve the precipitate in 2*N* NaOH and hydrolyze as in step 1. Carry 0.2 mL aliquots of the hydrolyzate forward to step 2.
2. Whole cells or other complex samples may need pretreatment, as described for the Burton assay for DNA (See Vol. 2). For example, the PCA/ethanol precipitate from extraction I may be used directly for the Lowry assay or the pellets remaining after the PCA hydrolysis (step 3 of the Burton assay) may be used for Lowry. In this latter case, both DNA and protein concentrations may be obtained from the same sample.
3. Rapid mixing as the Folin reagent is added is important for reproducibility.
4. A set of standards is needed with each group of assays, preferably in duplicate. Duplicate or triplicate unknowns are recommended.

References

1. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275.