



Content

Chapter I Spectrophotometry 1

He Chunyan, Wang Jihong, Du Fen, Zhu Ming'an, Tu Jiancheng

- 1.1 Basic Concepts and Principle of Spectrophotometry 1
- 1.2 Measurement of Light Absorption 4
- 1.3 Applications of Spectrophotometry 6
- EXP. 1 Quantitative Analysis of Protein 8
- EXP. 2 Determination of Blood Glucose 18

Chapter II Electrophoresis 23

Huang Xinxiang, He Chunyan, Hou Gan, Sheng Xiumei

- 2.1 Basic Principles of Electrophoresis 23
- 2.2 Impact Factors of Electrophoresis 24
- 2.3 Detection of Components after Electrophoretic Separation 26
- 2.4 Special Electrophoretic Techniques and Applications 27
- EXP. 3 Separation of Serum Proteins by Cellulose Acetate Membrane
Electrophoresis 31
- EXP. 4 Separation of Serum Lipoproteins by Agarose Gel Electrophoresis 35
- EXP. 5 Proteins Separation by SDS-Polyacrylamide Gel Electrophoresis 38

Chapter III Chromatography 44

Xiao Fangxiang, Yang Xuesong, Lin Chunrong, Yang Yanyan

- 3.1 Fundamental Principles of Chromatography 44
- 3.2 Commonly Used Chromatographic Techniques 46
- EXP. 6 Detecting Transamination of Amino Acids by Paper Chromatography 54
- EXP. 7 Separation of Mixed Amino Acids by Cation Exchange
Chromatography 58



EXP. 8 Separation of Hemoglobin and Dinitrophenyl (DNP)-glutamate by Gel Filtration Chromatography	62
--	----

Chapter IV Enzyme Analysis 65

Huang Dong'ai, Cai Wangwei, Sun Xuguo, Zhuo Shaoyuan

4.1 The Activity of Enzyme	65
4.2 Enzyme kinetics (Factors affecting reaction velocity)	67
4.3 Enzymatic analysis	70
EXP. 9 Assay the Activity of Alanine Aminotransferase (ALT) in Serum (Mohun's Method)	74
EXP. 10 Lactate Dehydrogenase (LDH) Analysis	78
EXP. 11 Effect of Substrate Concentration on Enzyme Activity-Determining Km Value for Alkaline Phosphatase	83
EXP. 12 Coupled enzymatic reaction assay (Enzymatic Endpoint Method) - Determination of Total Cholesterol, Triglycerides and Glucose in Serum	86

Chapter V Isolation and Purification of Protein 94

Yu Hong, Cao Jia, Guan Yaqun, Zhou Hongbo, Chen Yan

5.1 Methods and Basic Principles of Protein Purification	94
5.2 Procedure of Protein Preparation	100
5.3 Crystallization, Concentration, Drying and Storage of Protein	103
5.4 Identification and Analysis of Protein	104
EXP. 13 Isolation and Identification of Serum IgG	107
EXP. 14 Expression, Purification and Identification of Glutathione S-Transferase Fusion Protein	114

Chapter VI Isolation, Purification and Identification of Nucleic

Acids 121

Zhang Baifang, Yan Qiu, Ma Yongping, Zheng Fang

6.1 Isolation and Purification of Nucleic Acids	121
6.2 Identification and Analysis of Nucleic Acids	124
EXP. 15 Isolation of Eukaryotic Genomic DNA by Proteinase K-Phenol or NaI Method	132
EXP. 16 SDS-Alkaline Lysis of Plasmid DNA	138
EXP. 17 Isolation of Total RNA by TRIzol Reagent	141



EXP. 18	Identification of DNA by UV-Spectrophotometry and Gel Electrophoresis	145
EXP. 19	Identification of RNA by UV-Spectrophotometry and Formaldehyde Denaturing Agarose Gel Electrophoresis	153
EXP. 20	Polymerase Chain Reaction (PCR) and Reverse Transcription-PCR	157

Chapter VII Genetic Engineering

Ge Yinlin, Wu Junzhu, Bu Youquan, Tang Wei

7.1	The Process of Gene Cloning	163
7.2	Enzymes for Gene Cloning	165
7.3	Vectors for Gene Cloning	166
7.4	Expression of Foreign Genes and Purification of Recombinant Proteins	168
EXP. 21	DNA Cloning	171
EXP. 22	Expression of Exogenous Gene in <i>E. coli</i> and Purification of the Expressional Product	180

English-Chinese Index

190

1.1 Basic Concepts and Principle of Spectrophotometry

Light is a kind of electromagnetic wave. The light can be seen by our naked eyes is really a very small portion of the electromagnetic spectrum (Figure 1-1) within the wavelength range between approximately 380 nm and 760 nm. The visible light could be split into many colors by a prism. Each color is signed by the wavelength of light. Any solution that contains a substance that absorbs the visible light will appear a color. Light with wavelengths longer than 760 nm or shorter than 380 nm is invisible. Ultraviolet is a region of the electromagnetic spectrum that has a wavelength range from 375 nm to 12.5 nm. Ultraviolet-Visible spectrophotometry using lights in the visible range and adjacent near ultraviolet (UV) range (200 ~ 375 nm) is discussed in this chapter.

1.1.1 Absorption spectrum

Each substance has its own characteristic spectrum. The light absorption of the same substance at different wavelengths is different, and light absorption of different substances at the same wavelength is also different. Spectrophotometry is based on this selective absorption of