
Table of Contents

Abbreviations	XVII
1 Quantitative Methods	1
1.1 Quantitative Determinations of Proteins	1
1.1.1 LOWRY Protein Quantification	2
1.1.1.1 Standard Procedure	2
1.1.1.2 Modification by SARGENT	3
1.1.1.3 Micromethod on Microtest Plates	4
1.1.1.4 Protein Determination in the Presence of Interfering Substances	5
1.1.2 BRADFORD Protein Determination	6
1.1.3 Protein Determination in SDS-PAGE Sample Solutions	7
1.1.4 Protein Determination Using Amido Black	8
1.1.5 BCA Protein Determination	9
1.1.5.1 BCA Standard Procedure	9
1.1.5.2 BCA Micromethod	9
1.1.6 KJELDAHL Protein Determination	10
1.1.7 UV Photometric Assay of Protein Concentration	11
1.2 Quantitative Determination of Nucleic Acids	13
1.2.1 SCHMIDT and THANNHAUSER DNA, RNA, and Protein Separation Procedure	13
1.2.2 Orcin RNA (Ribose) Determination	14
1.2.3 Diphenylamine DNA (Deoxyribose) Determination	14
1.2.4 Quantitative DNA Determination with Fluorescent Dyes	15
1.2.5 Determination of Nucleic Acids by UV Absorption	16
1.3 Quantitative Phosphate Determinations	17
1.3.1 Determination of Inorganic Phosphate in Biologic Samples	17
1.3.2 Determination of Total Phosphate	18
1.3.3 Phospholipid Determination	18
1.4 Monosaccharide Determination	19
1.5 Calculations in Quantitative Analysis	20
2 Electrophoresis	23
2.1 Polyacrylamide Gel Electrophoresis Systems	23
2.1.1 LAEMMLI SDS-Polyacrylamide Gel Electrophoresis	26
2.1.2 SDS-Polyacrylamide Gel Electrophoresis at Neutral pH (NuPAGE)	31

2.1.3	SDS-Polyacrylamide Gel Electrophoresis According to WEBER, PRINGLE, and OSBORN	32
2.1.4	Urea-SDS-Polyacrylamide Gel Electrophoresis for the Separation of Low Molecular Weight Proteins	34
2.1.5	TRICINE-SDS-Polyacrylamide Gel Electrophoresis for Proteins and Oligopeptides in the Range of 1000–50 000 Daltons	35
2.1.6	SDS-Polyacrylamide Gel Electrophoresis at pH 2.4	36
2.1.7	Urea-Polyacrylamide Gel Electrophoresis for Basic Proteins at pH 2	37
2.1.8	Anodic Discontinuous Polyacrylamide Gel Electrophoresis (Native PAGE)	38
2.1.9	Cathodic Discontinuous Polyacrylamide Gel Electrophoresis (Native PAGE)	39
2.1.10	Affinity Electrophoresis	40
2.1.11	Two-Dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE; IEF followed by SDS-PAGE)	41
2.1.11.1	First Dimension: Isoelectric Focusing (IEF)	42
2.1.11.2	Second Dimension: SDS-PAGE (Acrylamide Gradient Gel)	44
2.2	Agarose and Paper Electrophoresis	45
2.2.1	Non-denaturing Nucleic Acid Electrophoresis	45
2.2.2	Denaturing Nucleic Acid Electrophoresis	46
2.2.3	Identification of Phosphoamino Acids (Paper Electrophoresis)	48
2.3	Aid in Electrophoresis	49
2.3.1	Marker Dyes for Monitoring Electrophoresis	49
2.3.1.1	Anodic Systems	49
2.3.1.2	Cathodic Systems	49
2.3.2	Marker Proteins for the Polyacrylamide Gel Electrophoresis	50
2.3.3	Covalently Colored Marker Proteins	52
2.4	Staining Protocols	53
2.4.1	Staining with Organic Dyes	53
2.4.1.1	Amido Black 10 B	54
2.4.1.2	Coomassie Brilliant Blue R250 and G250	54
2.4.1.3	Coomassie Brilliant Blue R250 Combined with Bismarck Brown R	55
2.4.1.4	Fast Green FCF	55
2.4.1.5	Stains All	56
2.4.1.6	Staining of Proteolipids, Lipids, and Lipoproteins	56
2.4.2	Silver Staining of Proteins in Gels	56
2.4.2.1	Citrate/Formaldehyde Development	57
2.4.2.2	Alkaline Development	58
2.4.2.3	Silver Staining Using Tungstosilicic Acid	58
2.4.2.4	Silver Staining of Proteins: Formaldehyde Fixation	59
2.4.2.5	Silver Staining of Glycoproteins and Polysaccharides	60
2.4.2.6	Enhancement of Silver Staining	60
2.4.2.7	Reducing of Silver-Stained Gels	61
2.4.3	Copper Staining of SDS-PAGE Gels	61

2.4.4	Staining of Glycoproteins and Polysaccharides in Gels	62
2.4.4.1	Staining with SCHIFF's Reagent (PAS Staining)	62
2.4.4.2	Staining with Thymol	63
2.4.5	Staining of Blotted Proteins on Membranes	63
2.4.5.1	Staining on Nitrocellulose with Dyes	63
2.4.5.2	Staining on Nitrocellulose with Colloidal Gold	64
2.4.5.3	Staining on PVDF Blotting Membranes with Dyes	65
2.5	Electroelution from Gels	66
2.5.1	Preparative Electroelution of Proteins from Polyacrylamide Gels	66
2.5.2	Removal of SDS	67
2.5.3	Electrotransfer of Proteins onto Membranes (Electroblotting; Western Blot): Semi-dry Blotting	68
2.5.4	Immunochemical Detection of Antigens After Electrotransfer (Immunoblotting)	70
2.5.4.1	Detection Using Horseradish Peroxidase (HRP)	72
2.5.4.2	Detection Using Alkaline Phosphatase (AP)	73
2.5.5	Chemiluminescence Detection on Blotting Membranes	74
2.5.5.1	Chemiluminescence Using HRP	74
2.5.5.2	Chemiluminescence Using AP	74
2.5.6	Carbohydrate-Specific Glycoprotein Detection After Electrotransfer	75
2.5.7	General Carbohydrate Detection on Western Blots	76
2.5.8	Affinity Blotting	77
2.5.9	Transfer of Nucleic Acids (SOUTHERN and Northern Blot)	78
2.6	Drying of Electrophoresis Gels	79
2.7	Autoradiography of Radioactive Labeled Compounds in Gels	80
3	Chromatography	83
3.1	Thin-Layer Chromatography	83
3.1.1	Identification of the N-terminal Amino Acid in Polypeptides (TLC of Modified Amino Acids	83
3.1.2	Thin-Layer Chromatography of Nucleoside Phosphates	85
3.1.3	Gradient Thin-Layer Chromatography of Nucleotides	85
3.1.4	Identification of Phosphates on TLC Plates	87
3.1.5	Lipid Extraction and TLC of Lipids	88
3.2	Hints for Column Chromatography of Proteins	89
3.3	Gel Permeation Chromatography (GPC; Gel Filtration, GF; Size-Exclusion Chromatography, SEC)	93
3.3.1	Selection of Supports	96
3.3.2	Filling of a Gel Filtration Column	97
3.3.3	Sample Application and Chromatographic Separation (Elution)	97
3.3.4	Cleaning and Storage	98
3.3.5	Determination of Void Volume V_0 and Total Volume V_t	99
3.3.6	Removing of Unbound Biotin After Conjugation by Gel Filtration ("Desalting")	99
3.4	Ion Exchange Chromatography (IEC)	102
3.4.1	Preparation of Ion Exchange Supports	103

3.4.2	Capacity Test	104
3.4.3	Sample Application	104
3.4.4	Elution	105
3.4.5	Cleaning and Regeneration	105
3.4.6	High-Performance Ion Exchange Chromatography (HPIEC) of Mono- and Oligosaccharides	106
3.5	Hydrophobic Interaction Chromatography (HIC)	107
3.5.1	Capacity Test	107
3.5.2	Elution	108
3.5.3	Regeneration	108
3.5.4	Analytical HPLC of Hapten-Protein Conjugates	108
3.6	Affinity Chromatography (AC)	109
3.6.1	Cyanogen Bromide Activation of Polysaccharide-Based Supports	113
3.6.1.1	Determination of the Degree of Activation	114
3.6.2	Coupling to Cyanogen Bromide-Activated Gels	114
3.6.2.1	Quantitative Determination of Coupled Diamine Spacers with 2,4,6-Trinitrobenzene Sulfonic Acid	115
3.6.2.2	Quantitative Determination of Immobilized Protein .	116
3.6.2.3	Immobilization of Wheat Germ Agglutinin	116
3.6.2.4	Affinity Purification of HRP	117
3.6.2.5	Affinity Chromatography of Immunoglobulins on Immobilized Antibodies (Immunoaffinity Chromatography, IAC)	117
3.6.2.6	Affinity Chromatography of Rabbit IgG on Protein-A Supports	118
3.6.3	Activation of Sepharose with Epichlorohydrin	119
3.6.3.1	Determination of Epoxy Residues	119
3.6.4	Immobilization of Monosaccharides (Fucose)	119
3.6.5	Activation with Divinylsulfone	120
3.6.6	Coupling of Reactive Dyes to Polysaccharides (Dye-Ligand Chromatography)	121
3.6.7	Covalent Coupling of Biotin (Biotin-Avidin/Streptavidin System)	121
3.6.8	Metal Chelate Chromatography of Proteins Containing His ₆ -Tag	123
3.7	Concentration of Diluted Protein Solutions	124
3.7.1	Acidic Precipitation	124
3.7.2	Salting Out	124
3.7.3	Precipitation Using Organic Substances	125
3.7.4	Lyophilization (Freeze Drying)	126
3.7.5	Ultrafiltration	127
4	Immunochemical Protocols	129
4.1	Conjugation of Haptens (Peptides) to Carrier Proteins	129
4.1.1	Activation of Proteins with TRAUT's Reagent Yielding Proteins with Additional Free SH Groups	132
4.1.2	Conjugation of MCA-Gly Peptides to SH-Carrying Proteins	132

4.1.3	Conjugation of Sulfhydryl Peptides Using 4-(N-Maleimidomethyl)-Cyclohexane-1-Carboxylic Acid N-Hydroxysuccinimide Ester (SMCC)	133
4.1.4	β -Galactosidase-Immunoglobulin Conjugate (Coupling via SH Groups)	134
4.1.4.1	Enzyme Reaction of β -Galactosidase	134
4.1.5	Carbodiimide Coupling of Peptides to Carrier Proteins with 1-Ethyl-3-(3-Dimethylaminopropyl)-Carbodiimide (EDAC, EDC)	134
4.1.6	Conjugation of Horseradish Peroxidase (Glycoproteins) by Periodate Oxidation	135
4.1.7	Conjugation of Peptides to Carrier Proteins Using Glutaraldehyde (Two-Step Procedure)	136
4.1.8	Conjugation of HRP to Antibodies with Glutaraldehyde	137
4.1.9	Alkaline Phosphatase-Immunoglobulin Conjugate (Glutaraldehyde Protocol)	138
4.1.9.1	Enzymatic Reaction of Alkaline Phosphatase from Calf Intestine	138
4.1.10	Labeling of Immunoglobulins with Fluorescent Dyes	138
4.1.11	Protein-Colloidal Gold Conjugates	141
4.1.11.1	Preparation of Colloidal Gold Sol	141
4.1.11.2	Adsorption of Protein to Colloidal Gold	142
4.2	Immunization of Laboratory Animals	143
4.3	Ammonium Sulfate Fractionation of Immunoglobulins	144
4.4	Removal of Unspecific Immunoreactivities	146
4.4.1	Preparation of Tissue Powder (Liver Powder)	148
4.5	Preparation of Egg Yolk IgY Fraction	148
4.6	Antibody Fragmentation	149
4.6.1	F(ab') ₂ Fragments from IgG	149
4.6.2	Fab' Fragments (Rabbit)	150
4.6.3	Fab Fragments (Rabbit)	150
4.7	HEIDELBERGER Curve (Precipitin Curve)	150
4.8	OUCHTERLONY Double-Radial Immunodiffusion	151
4.8.1	Purification of Agar	151
4.8.2	Preparation of Slides	151
4.8.3	Immunodiffusion	152
4.8.4	Visualization of the Precipitin Lines	152
4.9	Immunoprecipitation of Antigens	153
4.10	Immuno-electrophoresis	154
4.11	Counter-electrophoresis	155
4.12	Dot-Blot Assay	156
4.13	Enzyme Immunosorbent Assay (EIA, ELISA)	157
4.13.1	Indirect EIA with HRP Conjugate	158
4.13.2	Determination of Enzyme Activity by ELISA	159
4.13.3	Isotype Determination by EIA (AP Conjugate)	160

5	Centrifugation	161
5.1	Speed vs Centrifugal Force Graphs	161
5.2	Differential Centrifugation	164
5.3	Density Gradient Centrifugation	165
5.3.1	Pre-formed Discontinuous Gradient Centrifugation: Isolation of Liver Cell Nuclei	166
5.3.2	Sucrose Gradient Centrifugation: Preparation of Surface Membranes (Sarcolemma, SL) of Heart Muscle Cells	167
5.3.2.1	Determination of a Marker Enzyme: Ouabain-Sensitive Na,K-ATPase	172
5.3.2.2	Receptor Determination: DHP Binding Sites on Surface Membranes	173
5.3.2.3	Determination of the Dissociation and Association Kinetics of the DHP Receptors	174
5.3.3	RNA Separation by Non-Denaturing Sucrose Density Gradient Centrifugation	175
5.3.4	Denaturing RNA Gradient Centrifugation	176
5.3.5	Isopycnic Centrifugation	177
5.3.5.1	Purification of High Molecular Weight DNA in CsCl Gradients	177
5.3.5.2	Cell Fractionation Using Percoll	178
5.3.5.3	Preparation of Human Lymphocytes	179
6	Radioactive Labeling	181
6.1	Radioactive Decay	182
6.2	Decay Tables for ³² P-Phosphorus, ³⁵ S-Sulfur, and ¹²⁵ I-Iodine	183
6.3	Enzymatic [³² P]-Phosphate Incorporation into Proteins	185
6.4	Iodination with [¹²⁵ I]-Iodine Reagents	187
6.4.1	Chloramine-T Protocol	187
6.4.2	Iodination with BOLTON-HUNTER Reagent	188
6.5	Scintillation Cocktails for Liquid Scintillation Counting	188
7	Buffers	191
7.1	Theoretical Considerations	191
7.2	Plot for Buffer Calculations	198
7.3	pH Indicators	199
7.4	Buffer Recipes	199
7.4.1	Commonly Used Buffers	201
7.4.2	Buffers and Media for Tissue and Cell Culture and Organ Perfusion	204
7.4.3	pH Calibration Buffers	206
7.4.4	Volatile Buffers	207
8	Tables	209
8.1	Concentration Units	209
8.2	Conversion Factors for SI Units	210
8.3	Data of Frequently Used Substances	212
8.4	Protein Data	216

8.5	Protease Inhibitors	221
8.6	Single-Letter Codes and Molecular Masses of Amino Acids	222
8.7	Spectroscopic Data of Nucleotides	225
8.8	Detergents (“Surfactants”)	225
8.9	Refractive Index and Density of Sucrose Solutions	228
8.10	Ammonium Sulfate Saturation Table	229
8.11	Diluted Solutions	231
8.12	Mixture Rule	232
9	Statistics and Data Analysis	233
9.1	Statistical Equations	233
9.1.1	Mean and Related Functions	233
9.1.2	Correlation: Linear Regression	234
9.1.3	The <i>t</i> -test (Student’s Test)	236
9.2	Data Analysis	237
9.2.1	Receptor–Ligand Binding	237
9.2.2	Enzyme Kinetics	240
9.2.3	Determination of Molecular Mass by SDS-PAGE	243
9.3	Diagnostic Sensitivity and Specificity	244
9.4	Software for the Lab	244
9.4.1	Data Analysis and Presentation	245
9.4.2	Software for Statistics	245
9.4.3	Other Software	245
9.4.4	Selected Internet Links	246
	Subject Index	247



<http://www.springer.com/978-3-540-32785-1>

Basic Methods for the Biochemical Lab

Holtzhauer, M.

2006, XVIII, 252 p. 23 illus., 1 illus. in color., Softcover

ISBN: 978-3-540-32785-1