

Contents

1	General Aspects of Nonradioactive Labeling and Detection (C. Kessler)	1
1.1	Introduction	1
1.2	The Concept of Nonradioactive Bioanalytics	2
1.3	Direct and Indirect Labeling and Detection Systems	4
1.3.1	Direct Systems	5
1.3.2	Indirect Systems	5
1.4	Labeling Methods	11
1.4.1	Enzymatic Labeling	12
1.4.2	Photochemical Labeling	13
1.4.3	Chemical Labeling	13
1.5	Detection Methods	16
1.5.1	Direct Detection Systems	16
1.5.2	Indirect Detection Systems	18
1.5.2.1	Optical Detection	18
1.5.2.2	Luminescence Detection	19
1.5.2.3	Fluorescence Detection	20
1.6	Guide of the Use of Information in this Book	23
I	Standard Nonradioactive Labeling and Detection Systems	
2	Overview of Nonradioactive Labeling Systems (C. Kessler)	27
3	The Digoxigenin: Anti-Digoxigenin (DIG) System	35
3.1	Overview (C. Kessler)	35
3.2	Labeling and Detection of Nucleic Acids (H.-J. Höltke, R. Seibl, G. G. Schmitz, T. Walter, R. Rüger, G. Sagner, J. Burg, K. Mühlegger, and C. Kessler)	36
3.2.1	Principle and Applications	36

3.2.2	Reaction Scheme	38
3.2.3	Random-Primed Labeling with DIG-[11]-dUTP and Klenow Enzyme	38
3.2.4	PCR-Guided Synthesis of Vector-Free, DIG-Labeled DNA Probe with DIG-[11]-dUTP	40
3.2.5	RNA Labeling by Run-off Transcription with DIG-[11]-UTP and SP6, T7 or T3 RNA Polymerase	42
3.2.6	Oligonucleotide Tailing with DIG-[11]-dUTP/dATP and Terminal Transferase	43
3.2.7	Oligonucleotide 3' End Labeling with DIG-[11]-ddUTP and Terminal Transferase	45
3.2.8	Photolabeling of DNA or RNA with PhotoDIG	46
3.2.9	Hybridization with DIG-Labeled DNA or DIG-Labeled Oligonucleotides	46
3.2.10	Hybridization with DIG-Labeled RNA	49
3.2.11	Optical Detection of DIG Modification with BCIP/NBT	50
3.2.12	Chemiluminescent Detection of DIG Modification with Lumigen TM PPD or Lumiphos TM 530	51
3.2.13	Special Hints for Application and Troubleshooting	53
3.3	Labeling and Detection of Proteins and Glycoproteins (A. Haselbeck and W. Hösel)	56
3.3.1	Principle and Applications	56
3.3.2	Labeling and Detection of Proteins/Peptides	56
3.3.3	Labeling and Detection of Glycoconjugates	57
3.3.4	General Labeling and Detection of Proteins/ Peptides with DIG-Ester and DIG-Maleimide	57
3.3.5	Selective Labeling of Sulfhydryl Groups	60
3.3.6	Selective Labeling of Disulfides	61
3.3.7	Special Hints for Application and Troubleshooting	61
3.3.8	General Labeling and Detection of Glycoconjugates with DIG-Hydrazide	62
3.3.9	Special Hints for Application and Troubleshooting	64
3.3.10	Sialic Acid-Specific Oxidation	65
3.3.11	Special Hints for Application and Troubleshooting	66
3.3.12	Selective Detection of Terminal Galactose Residues	66
3.3.13	Special Hints for Application and Troubleshooting	66
3.3.14	Labeling of Glycoconjugates with DIG-Lectins	67
3.3.15	Special Hints for Application and Troubleshooting	68
4	The Biotin System	70
4.1	Labeling and Detection of Nucleic Acids (A. Rashtchian and J. Mackey)	70
4.1.1	Principle and Applications	70

	Contents	XI
4.1.1.1	Labeling of Nucleic Acids with Biotin	71
4.1.1.2	Biotinylated Nucleotides	71
4.1.2	Methods of Labeling DNA	73
4.1.2.1	Biotin Labeling of DNA by Nick Translation	73
4.1.2.2	Random Primer Labeling of DNA with Biotin	75
4.1.2.3	Polymerase Chain Reaction	77
4.1.2.4	Labeling with Photobiotin	79
4.1.3	Labeling of Synthetic Oligonucleotides	80
4.1.3.1	Enzymatic Labeling	80
4.1.3.2	Chemical Synthesis of Biotinylated Oligonucleotides	82
4.1.4	Methods of Labeling RNA	82
4.1.5	Hybridization and Detection of Biotinylated Probes	84
4.1.5.1	Hybridization	85
4.1.5.2	Hybridization of Synthetic Oligonucleotide Probes	86
4.1.5.3	Binding the Streptavidin-Alkaline Phosphatase Conjugate	87
4.1.5.4	Chromogenic Detection of Biotinylated Probes with Alkaline Phosphatase	87
4.1.5.5	Chemiluminescent Detection of Biotinylated Probes with Alkaline Phosphatase	88
4.1.6	Summary	88
4.2	Labeling and Detection of Proteins and Glycoproteins (E. A. Bayer and M. Wilchek)	91
4.2.1	Principle and Applications	91
4.2.2	Biotinylation of Proteins via Lysines	92
4.2.3	Special Hints for Application and Troubleshooting	93
4.2.4	Biotinylation of Proteins via Cysteines	94
4.2.5	Special Hints for Application and Troubleshooting	95
4.2.6	Biotinylation of Proteins via Tyrosines or Histidines	95
4.2.7	Special Hints for Application and Troubleshooting	96
4.2.8	Biotinylation of Proteins via Aspartatic and Glutamic Acids	96
4.2.9	Special Hints for Application and Troubleshooting	97
4.2.10	Biotinylation of Glycoproteins via Sugar Residues	98
4.2.11	Special Hints for Application and Troubleshooting	99
5	In Vivo Labeling of DNA Probes with 5-BrdU (J. L. Guesdon)	101
5.1	Principle and Applications	101
5.2	Reaction Scheme	103
5.3	In Vivo Recombinant M13 DNA Labeling with 5-BrdU, Hybridization, and Immunoenzymatic Detection	103
5.4	Special Hints for Application and Troubleshooting	107

6	The Sulfone System (I. Nur and M. Herzberg)	110
6.1	Principle and Applications	110
6.2	The Photo-ChemiProbe Procedure: Labeling by Sulfonation, Hybridization, and Chemiluminescence Visualization	111
6.2.1	BCIP/NBT as an Alternative Chromogenic System	113
6.3	Special Hints for Application and Troubleshooting	113
6.4	Labeling of Oligonucleotide Primers for Use in PCR and the Use of Amplified Products as a Labeled Probe	114
7	Colloidal Gold as a Marker in Molecular Biology: The Use of Ultra-Small Gold Probes (P. F. E. M. van de Plas and J. L. M. Leunissen)	116
7.1	Principle and Applications	116
7.1.1	Limitations of the Gold Marker System	117
7.1.2	Improvement by Reduction of Particle Size	117
7.2	Reaction Scheme	118
7.3	Indirect Immunogold Silver Staining	119
7.4	Special Hints for Application and Troubleshooting	121
7.5	Selected Example: Immunodetection of Gonadatropic Hormone in Catfish Pituitary	123
8	Direct Peroxidase Labeling of Hybridization Probes and Chemiluminescence Detection (I. Durrant)	127
8.1	Principle and Applications	127
8.2	Reaction Scheme	129
8.3	Directly Labeled Nucleic Acid (Long) Probes	130
8.4	Oligonucleotide Probes	131
8.5	Special Hints for Application and Troubleshooting	132
9	A Highly Sensitive Method for Detecting Peroxidase in In Situ Hybridization or Immunohistochemical Assays (J. G. Lazar and F. E. Taub)	135
9.1	Principle and Applications	135
9.2	Reaction Scheme	138
9.3	In Situ Detection of Peroxidase	138
9.4	Special Hints for Application and Troubleshooting	141
10	The SNAP System (J. E. Marich and J. L. Ruth)	143
10.1	Principle and Applications	143
10.2	Colorimetric Detection of <i>Mycobacteria</i> on Filters	144

10.3	Colorimetric Detection of Human Papillomavirus In Situ	145
10.4	Chemiluminescent Detection of Human DNA Fingerprints	146
10.5	Fluorescence Detection of Human Immunodeficiency Virus Using Sandwich Hybridization	147
II	Specialized Nonradioactive Detection Systems	
11	Overview of Colorimetric, Chemiluminometric, and Fluorimetric Detection Systems (H.-J. Guder and H.-P. Josel)	153
11.1	Principle and Applications	153
11.2	Substrates for Hydrolases	154
11.2.1	Direct Substrates	154
11.2.2	Indirect Substrates	155
11.3	Substrates for Peroxidase	155
11.4	Applications of Direct Labels	156
12	Colorimetric Systems	159
12.1	Indigo/Tetrazolium Dyes (H.-J. Guder)	159
12.1.1	Principle and Applications	159
12.1.2	Detection with BCIP/NBT	161
12.2	Azo Dyes (W. Kunz and S. West)	161
12.2.1	Principle and Applications	161
12.2.2	Detection with Azo Dyes	163
12.2.3	Special Hints for Application and Troubleshooting	164
13	Luminescence Systems	165
13.1	Chemiluminescence: Luminol (H.-P. Josel)	165
13.1.1	Principle and Applications	165
13.1.2	Chemiluminescent Labeling and Detection with Horseradish Peroxidase and Luminol	166
13.1.3	Special Hints for Application and Troubleshooting	167
13.2	Chemiluminescence: Properties of 1,2-Dioxetane Chemiluminescence (I. Bronstein and L. J. Kricka)	168
13.3	Electrochemiluminescence: Ruthenium Complexes (J. H. Kenten)	175
13.3.1	Principle and Applications	175
13.3.2	Detection of PCR Products Using an Electrochemiluminescence Assay	176
13.3.3	Special Hints for Application and Troubleshooting	178

13.4	Bioluminescence: Luciferin (R. E. Geiger and E. Schneider)	179
13.4.1	Principle and Applications	179
13.4.2	Reaction Scheme	180
13.4.3	Bioluminescence-Enhanced Detection	181
13.4.3.1	Enzymatic Labeling with Alkaline Phosphatase	181
13.4.3.2	Enzymatic Labeling with β -Galactosidase	182
13.4.4	Special Hints for Application and Troubleshooting	182
14	Fluorescence Systems	185
14.1	Labeling of Biomolecules with Fluorescein, Resorufin, and Rhodamine (H.-P. Josel)	185
14.1.1	Principle and Applications	185
14.1.2	Fluorescent Labeling of Proteins	187
14.1.3	Special Hints for Application and Troubleshooting	188
14.2	Time-Resolved Fluorescence (E. P. Diamandis and T. K. Christopoulos)	188
14.2.1	Principle and Applications	188
14.2.2	Reaction Scheme	189
14.2.3	DNA Labeling with Biotin, Hybridization, and Detection	189
14.2.4	Special Hints for Application and Troubleshooting	191
III	Enhanced Systems	
15	Overview of Amplification Systems (C. Kessler)	197
15.1	Introduction	197
15.2	Target Amplification	197
15.2.1	Replication	197
15.2.2	Transcription	200
15.3	Signal Amplification	200
15.3.1	Coupling of the Binding Partners	201
15.3.2	Antibody Trees and Probe "Xmas Trees"/ Probe "Brushes"	201
15.3.3	Enzyme/Polyenzyme Amplification	202
15.3.4	Coupled Signal Cascades	202
15.4	Target-Specific Signal Amplification	202
15.4.1	Replication	202
15.4.2	Selective Complex Separation	202
15.5	In Vivo Amplification	203

16	Enhanced Signal Generation by Target Amplification	206
16.1	Polymerase Chain Reaction Amplification (R. Rüger)	206
16.1.1	Principle and Applications	206
16.1.2	DIG Labeling by PCR Amplification	209
16.1.3	Further Processing of DIG-Labeled PCR Products	210
16.1.4	Special Hints for Application and Troubleshooting	211
16.2	Amplification of Nucleic Acid Sequences by the Repair Chain Reaction (D. Segev)	212
16.2.1	Principle and Applications	212
16.2.2	Detection of HPV16 in Caski Cell Line DNA and in Clinical Biopsy Targets	212
16.2.3	Summary	217
16.3	Isothermal Amplification of DNA Targets by Strand Displacement Amplification (M. C. Little, J. G. Nadeau, G. T. Walker, J. L. Schram, M. S. Fraiser, A. Alexander, and D. P. Malinowski)	218
16.3.1	Principle and Applications	218
16.3.2	Reaction Scheme	220
16.3.3	Strand Displacement Amplification	220
16.3.4	Special Hints for Application and Troubleshooting	222
16.4	Ligase Chain Reaction (G. H. Shimer Jr. and K. C. Backman)	223
16.4.1	Principle and Applications	223
16.4.2	Ligase Chain Reaction Assay	225
16.4.3	Nonradioactive Detection of Amplification Products	227
16.5	Isothermal Target Amplification (E. James)	228
16.5.1	Principle and Applications	228
16.5.2	Nucleic Acid Sequence Based Amplification: General Procedure	231
16.5.3	Special Hints for Application and Troubleshooting	232
16.6	The Potential of rDNA in Identification and Diagnostics (E. Stackebrandt and W. Liesack)	232
16.6.1	Principle and Applications	232
16.6.2	Isolation of Genomic DNA from Pure Cultures	234
16.6.3	Isolation of Genomic DNA from Mixed Cultures and Environmental Samples	235
16.6.4	PCR-Mediated Amplification of 16S rDNA	235
16.6.5	Amplification of 16S rDNA from Genes	235

17	Signal Amplification System: Substrate Cascade (R. I. Carr, F. K. Wong, and D. Sadi)	240
17.1	Principle and Applications	240
17.2	Amplification of Alkaline Phosphatase Substrates	240
17.3	Special Hints for Application and Troubleshooting	241
IV	Application Formats	
18	Overview of Application Formats (C. Kessler)	247
19	Blot Formats: Nucleic Acids	253
19.1	Factors Influencing Nucleic Acid Hybridization (C. Kessler)	253
19.2	Dot, Southern, and Northern Blots (B. Rüger and C. Kaletta)	257
19.2.1	Principle and Applications	257
19.2.2	Preparation of Southern and Northern Blots	257
19.2.3	Southern Blot Hybridization	259
19.2.3.1	Special Aspects of Southern Blot Hybridization	262
19.2.4	Special Hints for Application and Troubleshooting	263
19.2.5	Northern Blot Hybridization	264
19.2.6	Special Hints for Application and Troubleshooting	265
19.2.7	Dot Blot Hybridization	265
19.2.8	Hybridization with Oligonucleotides in Southern and Northern Blotting	265
19.2.8.1	Hybridization in Tetramethylammonium Chloride	266
19.3	Colony and Plaque Hybridization (T. Walter)	267
19.3.1	Principle and Applications	267
19.3.2	Methods	267
19.3.3	Special Hints for Application and Troubleshooting	269
19.3.4	Example	269
19.4	Multilocus DNA Fingerprinting Using Nonradioactively Labeled Oligonucleotide Probes Specific for Simple Repeat Elements (J. T. Epplen and J. Máthé)	271
19.4.1	Principle and Applications	271
19.4.2	Demonstration of DNA Fingerprints by DIG-Labeled Oligonucleotides	273
19.4.3	Special Hints for Application and Troubleshooting	276
19.5	Tn5cos Restriction Mapping of Large DNA Plasmids (U. Zuber and W. Schumann)	277
19.5.1	Principle and Applications	277
19.5.2	Reaction Scheme	278
19.5.3	Tn5cos Restriction Mapping	278

19.6	DIG DNA Sequencing with Chemiluminescent or Dye Substrate (G. Sagner)	281
19.6.1	Principle and Applications	281
19.6.2	DIG DNA Sequencing and Detection	282
19.6.3	Special Hints for Application and Troubleshooting	287
19.7	DNA Sequencing: Chemiluminescent Detection with the 1,2-Dioxetane CSPD (C. S. Martin and I. Bronstein)	288
19.7.1	Principle and Applications	288
19.7.2	DNA Sequencing and Detection with CSPD	289
19.7.3	Special Hints for Application and Troubleshooting	292
19.8	Direct Blotting Electrophoresis for DNA Sequencing (T. M. Pohl)	293
19.8.1	Principle and Applications	293
19.8.2	DNA Sequencing by Direct Blotting Electrophoresis	294
20	Blot Formats: Proteins and Glycoproteins	297
20.1	Detection of Proteins and Glycoproteins on Western Blots (A. Haselbeck and W. Hösel)	297
20.1.1	Detection of Proteins	297
20.1.2	Detection of Glycoproteins	298
20.2	Southwestern Analysis Using DIG (S. Dooley)	299
20.2.1	Principle and Applications	299
20.2.2	DIG Southwestern Analysis	299
20.2.3	Special Hints for Application and Troubleshooting	302
21	In Situ Formats	304
21.1	Virus Detection in Fixed Cells (E. Genersch, B. J. Heiles and R. Neumann)	304
21.1.1	Principle and Applications	304
21.1.2	Detection of Viral Sequences in Fixed Cells and Tissues by In Situ Hybridization	305
21.1.3	Special Hints for Application and Troubleshooting	306
21.2	Differentiation of Viral and Chromosomal Nucleic Acids in Individual Nuclei (C. S. Herrington)	307
21.2.1	Principle and Applications	307
21.2.2	Non-Fluorescent In Situ Hybridization	309
21.2.3	Special Hints for Application and Troubleshooting	314
21.3	Virus Detection in Biopsy Specimens (P. Heino and V. Hukkanen)	316
21.3.1	Principle and Applications	316
21.3.2	Reaction Scheme	317

21.3.3	In Situ Hybridization of Tissue Specimens with DIG-[11]-dUTP-Labeled DNA and with DIG-[11]-UTP-Labeled RNA Probes . . .	317
21.3.4	Special Hints for Application and Troubleshooting	322
21.4	Fluorescent In Situ Hybridization on Banded Chromosomes (N. Arnold, M. Bhatt, T. Ried, J. Wienberg and D. C. Ward)	324
21.4.1	Principle and Applications	324
21.4.2	Detailed Standard Procedure	325
21.4.3	General Protocol	327
21.4.4	Chromosome Banding Protocols	329
21.5	Probe Labeling and Hybridization in One Step (J. Koch)	336
21.5.1	Principle and Applications	336
21.5.2	Procedure	338
21.5.3	Special Hints for Application and Troubleshooting	340
21.6	Multiple Fluorescence In Situ Hybridization for Molecular Cytogenetics (A. K. Raap, J. Wiegant, and P. Lichter)	343
21.6.1	Principle and Applications	343
21.6.2	Reaction Scheme	344
21.6.3	Multiple Fluorescence In Situ Hybridization	344
21.6.4	Special Hints for Application and Troubleshooting	351
21.7	Mapping of Polytene Chromosomes (E. R. Schmidt)	354
21.7.1	Principle and Applications	354
21.7.2	Mapping of Polytene Chromosomes by Multiple In Situ Hybridization	355
21.7.3	Special Hints for Application and Troubleshooting	357
21.8	Detection of mRNA in Fixed Cells with DIG-Labeled RNA Probes (A. Starzinski-Powitz and K. Zimmermann)	359
21.8.1	Principle and Applications	359
21.8.2	DIG-Labeled RNA Probes in the Detection of mRNA in Fixed Cells	360
21.9	Detection of mRNA in Fixed Tissues Using RNA Probes (K. J. Hillan)	363
21.9.1	Principle and Applications	363
21.9.2	Standard Procedure	364
21.9.3	Special Hints for Application and Troubleshooting	366
21.10	Detection of mRNA In Situ with DIG-Labeled Synthetic Oligonucleotide Probes (F. Baldino Jr., E. Robbins, and M. E. Lewis)	367

21.10.1	Principle and Applications	367
21.10.2	DIG-Labeled Synthetic Oligonucleotide Probes to Detect mRNA In Situ	369
21.10.3	Results and Concluding Remarks	372
21.11	Whole Mount In Situ Hybridization for the Detection of mRNA in <i>Drosophila</i> Embryos (D. Tautz)	373
21.11.1	Principle and Applications	373
21.11.2	Whole Mount In Situ Hybridization	374
21.11.3	Special Hints for Application and Troubleshooting	376
21.12	DIG-Labeled Single-Stranded DNA Probes for In Situ Hybridization (N. H. Patel and C. S. Goodman)	377
21.12.1	Principle and Applications	377
21.12.2	DIG-Labeled Single-Stranded DNA Probes	378
21.12.3	Special Hints for Application and Troubleshooting	380
21.13	Double Labeling of mRNA and Proteins in <i>Drosophila</i> Embryos (B. Cohen and S. M. Cohen)	382
21.13.1	Principle and Applications	382
21.13.2	Choice of Signal Detection System	383
21.13.3	Double Labeling of mRNA and Proteins	384
21.13.3.1	Whole Mount In Situ Hybridization	386
21.13.3.2	X-Gal Reaction Followed by In Situ Hybridization	388
21.13.3.3	In Situ Hybridization Followed by Antibody Staining	389
21.13.4	Special Hints for Application and Troubleshooting	390
22	Quantitative Formats	393
22.1	Affinity-Based Collection of Sandwich Hybrids: A Quantitative Hybridization Format (H. Söderlund and K. Korpela)	393
22.1.1	Principle and Applications	393
22.1.2	Quantification of HPV DNA by Affinity Capture	396
22.1.3	Special Hints for Application and Troubleshooting	397
22.2	Detection of DNA:RNA Target:Probe Complexes with DNA:RNA-Specific Antibodies (F. Coutlée, R. H. Yolken, and R. P. Viscidi)	399
22.2.1	Principle and Applications	399
22.2.2	Reaction Scheme	401
22.2.3	Detection of PCR-Amplified DNA by Enzyme Immunoassay	401
22.2.4	Special Hints for Application and Troubleshooting	405
22.3	Capturing of Displaced DNA Strands as a Diagnostic Method (M. Collins)	407
22.3.1	Principle and Applications	407
22.3.2	Strand Displacement Assay	409

22.4	Nonradiative Fluorescence Resonance Energy Transfer (R. A. Cardullo)	414
22.4.1	Basic Principles of Nonradiative Fluorescence Resonance Energy Transfer	414
22.4.2	Methodologies for Measuring Transfer Efficiencies	415
22.4.3	Using Energy Transfer to Monitor Nucleic Acid Hybridization	418
22.4.4	Summary	422
22.5	Colorimetric Assays with Streptavidin/Avidin-Coated Surfaces (R. Seibl and S. Koehler)	424
22.5.1	Principle and Applications	424
22.5.2	Quantitative Detection of DIG-Labeled Nucleic Acid	425
Appendix I Suppliers of Reagents		429
Appendix II Suppliers of Equipment		435