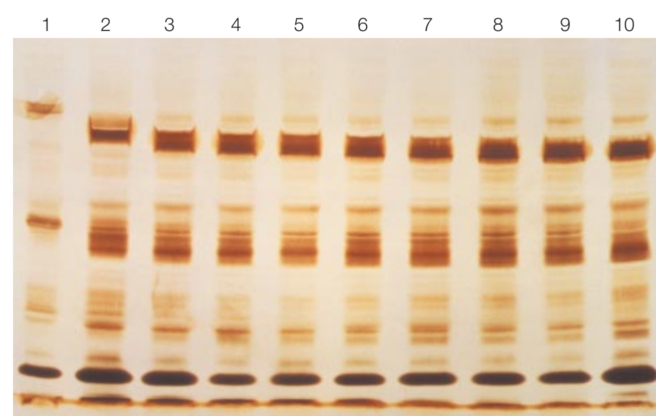
electrophoresis stains

The Bio-Rad Silver Stain

Introduction

Silver staining was first introduced as a general protein stain useful in polyacrylamide gel analysis in 1979 by Merril et al.[54] This first practical PAGE silver stain was of the silver diammine type adapted from early histological silver stains. In 1981, Merril et al.[55] introduced a faster, more reliable, and very sensitive silver stain derived from a photographic chemical process. Bio-Rad's silver stain is based on this photochemical method.



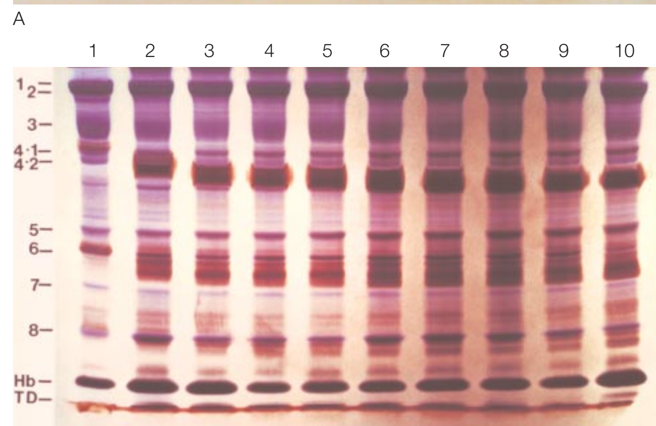


Fig. 1A and B. Differential and comprehensive visual detection of human red cell membrane proteins and sialoglycoproteins on gels stained with silver stain only (A) and with both silver/Coomassie Blue staining (B). Intact red cells were incubated with neuraminidase, and ghosts were isolated from samples taken at the indicated time intervals: 0, 5, 10 ,15, 20, 25, 30, 60, 80, and 120 min (lanes 1–10, respectively). 15 µl of packed ghost equivalents were applied to each well of an 11% (w/v) Laemmli gel.[39] Note the alteration in the silver stained pattern as a function of time. The silver stained bands correspond to the sialoglycoprotein bands. As can be seen, silver staining and Coomassie Blue staining are complementary, suggesting that the two stains detect different classes of macromolecules present in the gel.

The most important advantage of silver staining gels is the increased sensitivity obtained over other staining methods. Sensitivity is typically 50 times greater than obtained with classical Coomassie* Brilliant Blue R-250 staining. Increased sensitivity offers obvious advantages. For example, less sample is required when running gels. Also, analysis of dilute samples is possible. Protein purity can be assessed and contaminants detected more reliably. Silver stains detect a wider variety of macromolecules than does Coomassie Brilliant Blue R-250, including nucleic acids, glycoproteins, and lipoproteins.

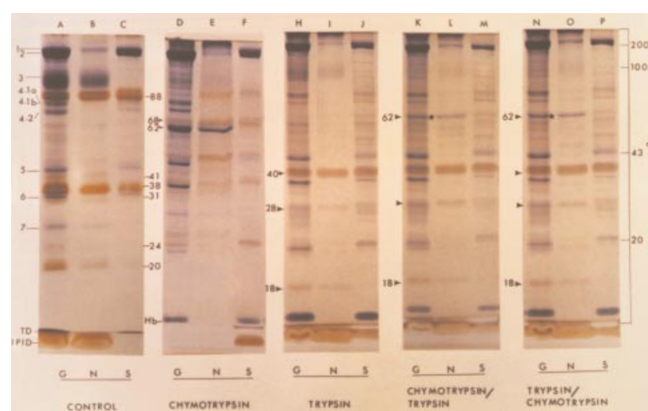


Fig. 2. Silver/Coomassie Blue double-stained gels of human erythrocyte ghosts, NaOH-stripped ghost pellets, and crude preparations of membrane sialoglycoproteins from untreated control and protease digested cells. Control cells were incubated without proteases (A–C). Test samples were erythrocytes treated with chymotrypsin (D–F), trypsin (H–J), or cells subjected to protocols of sequential digestion with chymotrypsin followed by trypsin (K-M) or trypsin treatment followed by chymotrypsin digestion (N–P).[18] Ghosts (G), NaOH-stripped ghost pellets (N), and crude isolates of the membrane sialoglycoprotein (S) fractions were prepared as described. Electrophoresis was performed on a 1.5 mm thick slab gel containing isotropic 11% (w/v) acrylamide with a stacking gel of 3% (w/v) acrylamide in the Laemmli buffer system.[39] 15 µl packed ghost equivalents were solubilized in Laemmli sample buffer and applied to each gel slot. After electrophoresis (12 hr at 25 mA), each gel slab was stained first with silver stain followed by Coomassie Blue staining.[17] Membrane sialoglycoproteins and lipids were stained yellow. The sialoglycoproteins migrated with estimated $M_r(10^{-3})$ = 88, 65, 41, 38, 31, 24 and 20 kD (control A, C). The 62,000 M_r chymotrypsin cleavage product of band 3 was stained blue with Coomassie Blue and is marked with (*). Yellow silver-stained chymotryptic cleavage products were observed at 47 and 68 kD (D–F). Trypsin cleavage products, which stained yellow with silver, were noted at 40, 28, and 18 kD. Figure 2 reprinted in color courtesy of Biochemical and Biophysical Research Communications.

Mechanism of Silver Staining

Mechanisms and features of silver staining have been discussed in prior reviews[51, 57]. The basic mechanism occurring in silver staining of macromolecules is the reduction of ionic to metallic silver. Protein bands are imaged in the gel due to differences in oxidation/reduction potentials between sites in gels occupied by protein and adjacent sites not occupied by protein. If protein-occupied sites have the higher reducing potential, then positive images are formed. Conversely, if sites unoccupied by protein have the higher reducing potential, then negative images are formed.

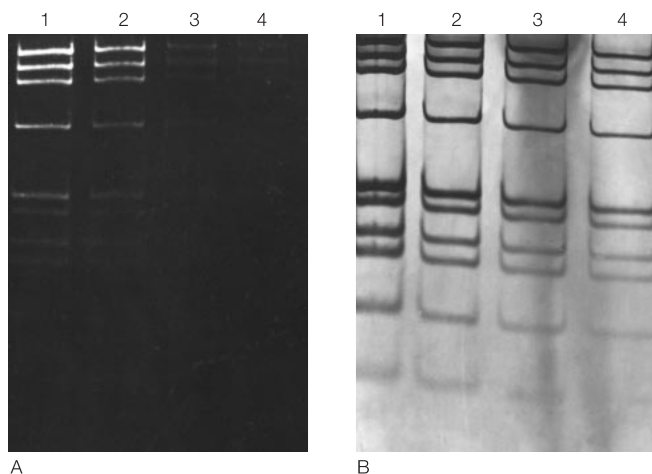


Fig. 3A and B. *Hae*III restriction digest of ØX174 RF DNA electrophoresed on a 0.75 mm 6% acrylamide slab gel containing Tris-borate-EDTA (TBE), pH 8.3 buffer. Amount of digest per lane: 1, 120 ng; 2, 50 ng; 3, 25 ng; 4, 10 ng; 5, 5 ng. Gels were stained with ethidium bromide (A), or with the Bio-Rad silver stain (B).

With photographic chemical-process silver stains, the silver nitrate reacts with protein sites under acidic conditions. Subsequent reduction of silver ions to metallic silver occurs by oxidation of formaldehyde under alkaline conditions. Sodium carbonate, or another base, buffers the formic acid produced by the oxidation of formaldehyde so that the silver ion reduction can continue until the protein bands appear in the gel.

With Bio-Rad's silver stain, the formation of a positive image is enhanced by dichromate oxidation, which may convert protein hydroxyl and sulfhydryl groups to aldehydes and thiosulfates, thereby altering the redox potential of the protein. Complexes formed between the proteins and dichromate may also form nucleation centers for silver reduction. Basic and sulfur-containing amino acids appear to be strongly involved in the formation of complexes with silver ions.[53] This also appears to be true for Coomassie stains. The incorporation of these amino acids into peptide chains, as well as cooperative effects of several intramolecular functional groups, are probably required for reaction with silver ions.[29] Reduction of ionic to metallic silver is highly dependent on pH. This reduction step is accomplished by the alkaline organic development reagent.

Before a protein gel can be stained, the proteins must be fixed to minimize diffusion of molecules in the gel. Fixation also elutes substances from the gel that are likely to interfere with establishment of the oxidation/reduction potential differences and with silver reduction. Ampholytes, detergents, reducing agents, initiators or catalysts, and buffer ions (glycine, chloride, etc.) must be removed. Good-quality acrylamide and bis, free of acrylic acid and metals, are required or background will be unacceptably high. Likewise, water used in all silver stain reactions must be of 1 µS conductance or less, and free of organic contaminants.

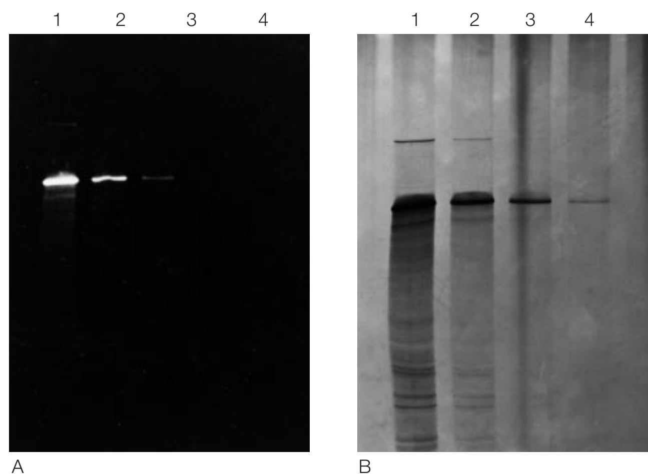


Fig. 4A and B. Sucrose gradient purified *E. coli* 16S ribosomal RNA electrophoresed on a 0.75 mm 4% acrylamide slab gel containing TBE buffer with 8 M urea. Amount of rRNA per lane: 1, 500 ng; 2, 100 ng; 3, 25 ng; 4, 5 ng. RNA sample provided by Doug Black and Dr Harry Noller, University of California, Santa Cruz. Gels were stained as described in Figure 3.

Abbreviated Procedure

An abbreviated procedure for Bio-Rad's silver stain is presented here, assuming a gel 0.75 to 1.0 mm thick. Times are longer for thicker gels and shorter for thinner gels. See the package insert for the complete procedure.

1. Fix in acid/alcohol	3 x 15 min with shaking
2. Oxidizer	5 min
3. Wash	10 min (until gel is clear)
4. Silver reagent	20 min
5. Wash	1–30 sec
6. Developer	1–10 min
	~90 min total

Photochemical vs. Silver Diammine Stains

Two silver stains are generally used: diammine, or ammoniacal, types,[54] and those adapted from photographic chemical development processes.[55] Silver diammine types require that gels be soaked first in basic silver diammine, followed by acid formaldehyde image development. Chemical stains require an initial gel soak in a weakly acidic silver nitrate solution, and development in alkaline formaldehyde. Prior to the silver nitrate step, gels are primed with a reducing agent such as dithiothreitol or an oxidizing reagent such as permanganate or dichromate. Dichromate (Bio-Rad) is most desirable, because images obtained have higher sensitivity and lowest background. Dithiothreitol generates signals of lower sensitivity, while permanganate generates higher backgrounds.

Diammine silver stains suffer from many disadvantages. Reagents are not stable and must be made fresh prior to staining. Reaction products are potentially explosive. Reaction times are lengthy in comparison to photochemical methods. Histochemical silver stains cannot be combined with Coomassie stains, fluorography, or autoradiography, and gels cannot be restained in order to visualize proteins not initially detected. Staining specificity is sensitive to variations in the concentration of silver ions relative to NaOH and NH_3, as well as to metals such as copper used in the stain, and to the acidification process used in image formation. Washes after silver diammine treatment are difficult, resulting in high background and variable sensitivity. Reproducibility and standardization of protein staining is therefore difficult.

By comparison, the photochemical method (Bio-Rad) requires only 3 reagents, which are stable on storage. It requires fewer reaction steps, and therefore is more rapid. This photochemical silver stain is sensitive, does not suffer from high background, is reproducible, and does not generate variable results when gels of different composition are stained.

Advantages of Silver Staining

Sensitive Alternative to Coomassie Blue R-250

- Dilute, unconcentrated samples can be analyzed
- Use of dilute sample avoids protein overload artifacts
- Trace samples can be analyzed
- Sample can be conserved

Useful Alternative to Autoradiography

- Faster and equally sensitive
- Less expensive
- May detect proteins not detected in radiolabeled cell lysate
- Does not require prohibitive amounts of radioactive precursors in whole animal studies
- Useful when autoradiography cannot be used

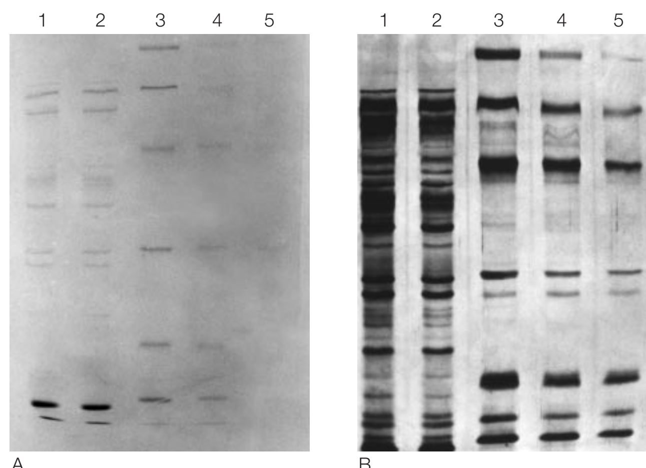


Fig. 5. SDS-PAGE according to Laemmli[39] stained with A, Coomassie Brilliant Blue R-250, and B, Bio-Rad's silver stain. Samples were run on 12% polyacrylamide gels 0.75 mm thick. Lane 1, bovine brain homogenate soluble fraction, 20 µl; lane 2, bovine brain homogenate soluble fraction, 10 µl; lane 3, Bio-Rad's SDS-PAGE low molecular weight standards, 1/40 dilution; lane 4, Bio-Rad's SDS-PAGE standards, 1/80; lane 5, Bio-Rad's SDS-PAGE standards, 1/160.

Features Unique to Photochemical Silver Stain

Simplicity

- Reliable. Most frequently quoted procedure
- Well characterized. Subject of multiple reviews[51, 56, 57]
- Few reagents — only 3, all stable at 4°C
- Rapid. <30 min for 0.5 mm gel, <45 min for 1 mm gel, <1.5 hr for 1.5 mm gel; combined mini-gel electrophoresis and stain complete in half a day

Specificity

- Stains all types of macromolecules including glycoproteins, lipoproteins, and nucleic acids (see References)
- Stains macromolecules not stained in other silver stain methods
- Recycling gel through silver nitrate and developer detects proteins not initially visualized; for example, calmodulin[51, 56, 57]

Universality

- Stains gels of various composition (see References) including IEF, 2-D, gradient and peptide gels, and nucleic acids in denaturing gels

Adjunct to Protein Identification and Characterization

- Protein-specific slopes (OD vs. protein concentration plot) differentiate one protein from another[57]
- Colors differentiate proteins

Compatibility with Other Detection Methods

- Can be followed by autoradiography, with ≤2% quenching[57, 81]
- Can perform fluorography, provided gel is destained[57, 81]
- Coomassie Blue R-250 following silver stain differentiates membrane proteins by color[16–19]
- Coomassie Blue R-250 before silver stain allows color differentiation,[41] reverses negative images, and enhances sensitivity[61]
- Coomassie Blue G-250 before silver stain increases sensitivity 2–8 times vs. silver stain alone[14]

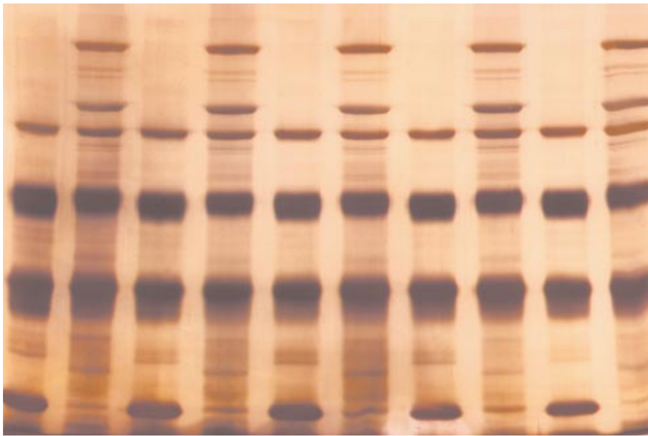


Fig. 6. Alternating high and low MW standard on a 0.75 mm gel, run at 100 V for 5 min and 200 V for 45 min in Bio-Rad's Mini-PROTEAN® II slab cell, and silver stained with Bio-Rad's silver stain kit.

Color Differentiation

- Differentiates membrane polypeptides (blue), lipid and sialoglycoprotein (yellow)[16–19]
- Differentiates polymorphisms[41]
- Color may be affected by charged amino side groups, bond length, and configuration[64]
- Color dependent on size of silver particle: small grains red or yellow-red, large grains blue to black[57]
- Color often enhanced by low concentration of reducing agent in developer, time of development, elevated temperature, or added alkali or metals in developer[57, 64]

Sensitivity

- Typically 50x more sensitive than Coomassie Blue R-250 (0.1 ng/mm^2)
- Can be as much as 200x more sensitive than Coomassie Blue R-250 (0.02 ng/mm^2)[57]
- Varies with protein
- Recycling or prior Coomassie staining may enhance sensitivity[14, 57, 61]

Quantitation and Linearity[57]

- Linear range 0.05–2 ng/mm^2
- Quantitative comparisons limited to homologous proteins
- Optical density vs. protein concentration plot generates protein-specific plots

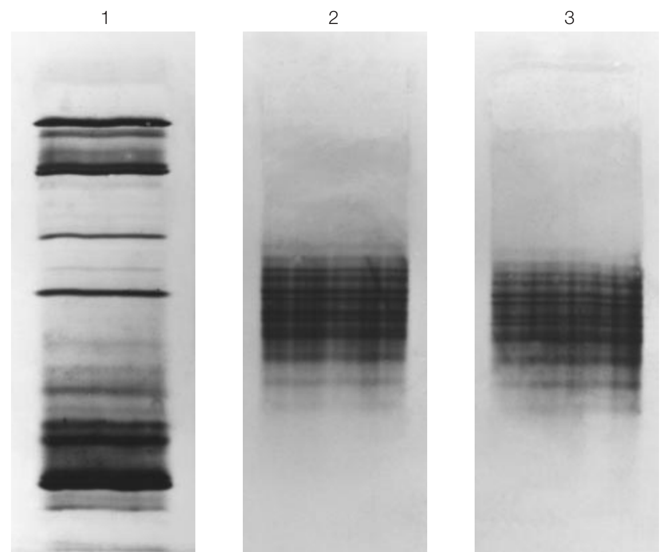


Fig. 7. Flatbed ultrathin IEF gel, pH 3–10, 5%T, 3%C stained with Bio-Rad's silver stain. Lane 1, Bio-Rad's IEF standards, is a mixture of 8 proteins, 4 of which are naturally colored; lanes 2 and 3 are goat IgG.

Nucleic Acid Stain

- More sensitive than ethidium bromide
  2–5x more sensitive (<0.03 ng/mm^2) for ss DNA[22]
  10–30x (<0.03 ng/mm^2) for RNA[48]
  100x (0.01 ng/mm^2) for ds DNA[3]
- Detects trace nucleic acids
- Detects small polynucleotides (10–20 bases)[3]; sensitivity 0.25–4 ng for DNA ≥271 bp
- Linear range 25–250 ng for DNA[22]

References, by Categories

Methodology	References
Photochemical silver staining	3–5, 9, 14–17, 22, 48–51, 55–57, 65, 67, 82
Coomassie G-250 stain prior to silver stain	15
Coomassie R-250 stain prior to silver stain	41, 61, 73
Coomassie R-250 stain after silver stain	14, 16–19
Multicolor silver staining	14, 16–19, 26, 41, 61, 64
Silver stain prior to fluorography	81
Silver stain prior to autoradiography	6, 81
Gradient gels	12, 26, 34, 73, 82
Two-dimensional PAGE	23, 26–28, 30–32, 36, 41, 50–52, 55–57, 58, 61, 62, 68, 72, 75, 79, 81
Isoelectric focusing	36, 49, 62
Peptide mapping/ proteolytic digests	17, 18, 32, 45
Quantitation	1, 15, 23, 31, 62, 75, 84
Recycling silver stain for image intensification	51, 56, 57
Negative staining and image reversal	57, 61

Macromolecules Stained	References
DNA/RNA	3, 4, 22, 48, 56
Glycoprotein	5, 40, 65, 71, 74, 86
Sialoglycoprotein	14, 16–19, 69
Carbohydrate	14, 46
Glycolipid	46
Lipoprotein	41, 75, 83
Lipid	16–19
Lipopolysaccharide	43
Membrane proteins	8, 16–19, 32, 72
Receptor proteins	1, 10, 11, 38, 46, 70, 71, 73
Nuclear proteins	3, 58
Total cellular proteins	18, 26, 30, 32, 36, 61, 62, 79, 81
Polypeptides	64
Hormones	15, 37
Oncogenic, growth, and cell factors	20, 24, 35, 44, 59, 63, 74, 82, 85

Applications	References
Assess blotting efficiency	7, 34, 80
Compare total protein in gels with blots	25, 32, 43, 60, 76, 80
Protein purity and integrity by gel analysis	10, 35, 37, 38, 47, 59, 73
Monitor reaction products	8
Assess purity of monoclonal antibodies	21, 77
Assess enzyme purity	12, 33, 37, 47, 60, 78, 84
Locate gel bands prior to band recovery	20
Compare total proteins to those radiolabeled in vivo or in vitro	6, 58
Assess purity and molecular weight of recombinant proteins	2, 40, 42, 79
Precursor-product relations	16
Topological analysis of membrane surfaces	16
Monitor chromatography fractions	
HPLC	21, 34, 59, 69, 74, 85
Affinity	7, 12, 24, 46, 68, 70, 73, 76, 86
Gel filtration/ion exchange	13, 20, 33, 42, 63, 82
Sucrose	65, 66
Clinical research	23, 26–28, 41, 50–52, 55–57, 61, 68, 75
Hormones and growth, cell, and oncogenic factors	20, 24, 35, 44, 59, 63, 74, 82, 85
Analysis of immune complexes	68
Mutation analysis	23, 79
Polymorphisms	23, 41
Analysis of cerebrospinal fluid	11, 27, 28, 49, 52, 55
Analysis of tumor cells	26, 61
Viral protein detection	7, 13, 66, 69, 76
Blood proteins	86

References

1 Acuto O et al., Purification and NH_2-terminal amino acid sequencing of the β subunit of a human T-cell antigen receptor, Proc Nat Acad Sci USA 81, 3851–3855 (1984)

2 Akiyoshi DE et al., Cloning and nucleotide sequence of the *tzs* gene from *Agrobacterium tumefaciens* strain T_{37}, Nucleic Acids Res 13, 2773–2788 (1985)

3 Beidler JL et al., Ultrasensitive staining of nucleic acids with silver, Anal Biochem 126, 374–380 (1982)

4 Berry MJ and Samuel CE, Detection of subnanogram amounts of RNA in polyacrylamide gels in the presence and absence of protein by staining with silver, Anal Biochem 124, 180–184 (1982)

5 Candiano G et al., Silver stain of proteins in ultra-thin gels containing carrier ampholytes — detection of glycosyl albumin with anionic and cationic charge in serums of diabetic patients, Clin Chim Acta 139, 195–201 (1984)

6 Chalbos D et al., Cloning of cDNA sequences of a progestin-regulated mRNA from MCF7 human breast cancer cells, Nucleic Acids Res 14, 965–982 (1986)

7 Chiou JF et al., Demonstration of a stimulatory protein for virus-specified DNA polymerase in phorbol ester-treated Epstein-Barr virus-carrying cells, Proc Natl Acad Sci USA 82, 5728–5731 (1985)

8 Christiaansen JE et al., Rapid covalent coupling of proteins to cell surfaces: immunological characterization of viable protein-cell conjugates, J Immunol Methods 74, 229–239 (1984)

9 Confavreux C et al., Silver stain after isoelectric focusing of unconcentrated cerebrospinal fluid: visualization of total protein and direct immunofixation of immunoglobulin G, Electrophoresis 3, 206–210 (1982)

10 Conti-Tronconi BM et al., Molecular weight and structural nonequivalence of the mature α subunits of *Torpedo californica* acetylcholine receptor, Proc Natl Acad Sci USA 81, 2631–2634 (1984)

11 Conti-Tronconi B et al., Brain and muscle nicotinic acetylcholine receptors are different but homologous proteins, Proc Natl Acad Sci USA 82, 5208–5212 (1985)

12 Coussen F et al., Identification of the catalytic subunit of brain adenylate cyclase: a calmodulin binding protein of 135 kDa, Proc Natl Acad Sci USA 82, 6736-6740 (1985)

13 Crooks AJ et al., The purification of alphavirus virions and subviral particles using ultrafiltration and gel exclusion chromatography, Anal Biochem 152, 295–303 (1986)

14 Deh ME et al., Sialoglycoproteins with a high amount of O-glycosidically linked carbohydrate moieties stain yellow with silver in sodium dodecyl sulfate-polyacrylamide gels, Anal Biochem 152, 166–173 (1985)

15 De Moreno MR et al., Silver staining of proteins in polyacrylamide gels: increased sensitivity through a combined Coomassie blue-silver stain procedure, Anal Biochem 151, 466–470 (1985)

16 Dzandu JK et al., Silver/Coomassie Blue double staining technique, Bio-Rad Laboratories, bulletin 1200

17 Dzandu JK et al., Detection of erythrocyte membrane proteins, sialoglycoproteins, and lipids in the same polyacrylamide gel using a double-staining technique, Proc Natl Acad Sci USA 81, 1733–1737 (1984)

18 Dzandu JK et al., A re-examination of the effects of chymotrypsin and trypsin on the erythrocyte membrane surface topology, Biochem Biophys Res Commun 126, 50–58 (1985)

19 Dzandu JK et al., Phosphorylation of glycophorin A in membranes of intact human erythrocytes, Biochem Biophys Res Commun 127, 878–884 (1985)

20 Fukada K, Purification and partial characterization of a cholinergic neuronal differentiation factor, Proc Natl Acad Sci USA 82, 8795–8799 (1985)

21 Gemski MJ et al., Single step purification of monoclonal antibody from murine ascites and tissue culture fluids by anion exchange high performance liquid chromatography, Biotechniques, 3, 378-384 (1985)

22 Goldman D and Merril CR, Silver staining of DNA in polyacrylamide gels: linearity and effect of fragment size, Electrophoresis 3, 24–26 (1982)

23 Goldman D et al., Lymphocyte proteins in Huntington's disease: quantitative analysis by use of two-dimensional electrophoresis and computerized densitometry, Clin Chem 28, 1021–1025 (1982)

24 Gospodarowicz D et al., Isolation of brain fibroblast growth factor by heparin-Sepharose affinity chromatography: identity with pituitary fibroblast growth factor, Proc Natl Acad Sci USA 81, 6963–6967 (1984)

25 Grove BK et al., A new 185,000-dalton skeletal muscle protein detected by monoclonal antibodies, J Cell Biol 98, 518–524 (1984)

26 Hanash SM et al., Lineage-related polypeptide markers in acute lymphoblastic leukemia detected by two-dimensional gel electrophoresis, Proc Natl Acad Sci USA 83, 807–811 (1986)

27 Harrington MG and Merril CR, Two-dimensional electrophoresis and "ultrasensitive" silver staining of cerebrospinal fluid proteins in neurological diseases, Clin Chem 30, 1933–1937 (1984)

28 Harrington MG et al., Two-dimensional electrophoresis of cerebrospinal fluid proteins in multiple sclerosis and various neurological diseases, Electrophoresis 5, 236–245 (1984)

29 Heukeshoven J and Dernick R, Simplified method for silver staining of proteins in polyacrylamide gels and the mechanism of silver staining, Electrophoresis 6, 103–112 (1985)

30 Heydorn WE et al., Effect of desmethylimipramine and reserpine on the concentration of specific proteins in the parietal cortex and the hippocampus of rats as analyzed by two-dimensional gel electrophoresis, J Pharmacol Exp Ther 229, 622–628 (1984)

31 Jacobowitz DM and Heydorn WE, Two-dimensional gel electrophoresis used in neurobiological studies of proteins in discrete areas of the rat brain, Clin Chem 30, 1996–2002 (1984)

32 Jahn R et al., A 38,000-dalton membrane protein (p38) present in synaptic vesicles, Proc Natl Acad Sci USA 82, 4137–4141 (1985)

33 Khan ZA and Fraenkel-Conrat H, Purification and characterization of polynucleotide phosphorylase from cucumber, Proc Natl Acad Sci USA 82, 1311–1315 (1985)

34 King SM et al., Characterization of monoclonal antibodies against *Chlamydomonas* flagellar dyneins by high-resolution protein blotting, Proc Natl Acad Sci USA 82, 4717–4721 (1985)

35 Kligman D and Marshak DR, Purification and characterization of a neurite extension factor from bovine brain, Proc Natl Acad Sci USA 82, 7136–7139 (1985)

36 Kondo I et al., Genetic analysis of human lymphocyte proteins by two-dimensional gel electrophoresis: VI. Identification of esterase D in the two-dimensional gel electrophoresis pattern of cellular proteins, Hum Genet 66, 248–251 (1984)

37 Kostyo JL et al., Biosynthetic 20-kilodalton methionyl-human growth hormone has diabetogenic and insulin-like activities, Proc Natl Acad Sci USA 82, 4250–4253 (1985)

38 Kull FC Jr et al., Cellular receptor for ^{125}I-labeled tumor necrosis factor: specific binding, affinity labeling, and relationship to sensitivity Proc Natl Acad Sci USA 82, 5756–5760 (1985)

39 Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227, 680–685 (1970)

40 Lee-Huang S, Cloning and expression of human erythropoietin cDNA in *Escherichia coli*, Proc Natl Acad Sci US, 81, 2708–2712 (1984)

41 Leren TP et al., Increased frequency of the apolipoprotein E-4 isoform in male subjects with multifactorial hypercholesterolemia, Clin Genet 27, 458–462 (1985)

42 Leung K and Chiao JW, Human leukemia cell maturation induced by a T-cell lymphokine isolated from medium conditioned by normal lymphocytes, Proc Natl Acad Sci USA 82, 1209–1213 (1985)

43 Linko-Kettunen L et al., Monoclonal antibodies to *Bacteroides fragilis* lipopolysaccharide, J Clin Microbiol 20, 519–524 (1984)

44 Ling N et al., Isolation and partial characterization of a M_r protein with inhibin activity from porcine follicular fluid, Proc Natl Acad Sci USA 82, 7217–7221 (1985)

45 Lischwe MA and Ochs D, A new method for partial peptide mapping using N-chlorosuccinimide/urea and peptide silver staining in sodium dodecyl sulfate-polyacrylamide gels, Anal Biochem 127, 453–457 (1982)

46 Maneckjee R, Purification and characterization of the mu opiate receptor from rat brain using affinity chromatography, Proc Natl Acad Sci USA 2, 594–598 (1985)

47 Markert M et al., Respiratory burst oxidase from human neutrophils: purification and some properties, Proc Natl Acad Sci USA 2, 3144–3148 (1985)

48 McNeilage LJ and Whittingham S, Use of the Bio-Rad silver stain to identify gel purified RNA components of small nuclear ribonucleoprotein antigens, J Immunol Methods 66, 253–260 (1984)

49 Mehta PD and Patrick BA, Detection of oligoclonal bands in unconcentrated CSF: Isoelectric focusing and silver staining, Neurology 33, 1365–1368 (1983)

50 Merril CR and Goldman, D, Quantitative two-dimensional protein electrophoresis for studies of inborn errors of metabolism, Clin Chem 28, 1015–1020 (1982)

51 Merril CR and Goldman D, Detection of polypeptides in two-dimensional gels using silver staining, pp 93–109 in Celis JE and Bravo R (eds) Two-Dimensional Gel Electrophoresis of Proteins. Methods and Applications, Academic Press, New York (1984)

52 Merril CR and Harrington MG, "Ultrasensitive" silver stains: their use exemplified in the study of normal human cerebrospinal fluid proteins separated by two-dimensional electrophoresis, Clin Chem 30, 1938–1942 (1984)

53 Merril CR and Pratt ME., A silver stain for the rapid quantitative detection of proteins or nucleic acids on membranes or thin layer plates, Anal Biochem 156, 96–110 (1986)

54 Merril CR et al., Trace polypeptides in cellular extracts and human body fluids detected by two-dimensional electrophoresis and a highly sensitive silver stain, Proc Natl Acad Sci USA 76, 4335–4339 (1979)

55 Merril CR et al., Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins, Science 211, 1437–1438 (1981)

56 Merril CR et al., Simplified silver protein detection and image enhancement methods in polyacrylamide gels, Electrophoresis 3, 17–23 (1982)

57 Merril CR et al., Gel protein stains: silver stain, Methods Enzymol 104, 441–447 (1984)

58 Miake-Lye R and Kirschner MW, Induction of early mitotic events in a cell-free system, Cell 1, 165–175 (1985)

59 Miyajima A et al., Secretion of mature mouse interleukin-2 by *Saccharomyces cerevisiae*: use of a general secretion vector containing promoter and leader sequences of the mating pheromone α-factor, Gene 37, 155–161 (1985)

60 Mogi M et al., Detection of inactive or less active forms of tyrosine hydroxylase in human adrenals by a sandwich enzyme immunoassay, Anal Biochem 38, 125–132 (1984)

61 Motté P, Two-dimensional electrophoretic analysis of cytosols from human breast tumors: optimal migration conditions, Clin Chem 30, 1947–1949 (1984)

62 Narayan RK, Proteins in normal, irradiated, and postmortem human brain quantitatively compared by using two-dimensional gel electrophoresis, Clin Chem 30, 1989–1995 (1984)

63 Neer EJ and Lok JM, Partial purification and characterization of a pp60$^{v\text{-}src}$-related tyrosine kinase from bovine brain, Proc Natl Acad Sci USA 82, 6025–6029 (1985)

64 Nielsen BL and Brown LR, The basis for colored silver-protein complex formation in stained polyacrylamide gels, Anal Biochem 141, 311–315 (1984)

65 Noll H et al., Characterization of toposomes from sea urchin blastula cells: a cell organelle mediating cell adhesion and expressing positional information, Proc Natl Acad Sci USA 82, 8062–8066 (1985)

66 Oesch B et al., A cellular gene encodes scrapie PrP 27-30 protein, Cell 40, 735–746 (1985)

67 Palumbo G and Tecce MF, A four- to sixfold enhancement in sensitivity for detecting trace proteins in dye or silver stained polyacrylamide gels, Anal Biochem 134, 254–258 (1983)

68 Per SR et al., Analysis of immune complexes by two-dimensional gel electrophoresis, Clin Immunol Immunopathol 34, 165–173 (1985)

69 Prusiner SB et al., Purification and structural studies of a major scrapie prion protein, Cell 38, 127–134 (1984)

70 Pytela R et al., A 125/115-kDa cell surface receptor specific for vitronectin interacts with the arginine-glycine-aspartic acid adhesion sequence derived from fibronectin, Proc Natl Acad Sci USA 82, 5766–5770 (1985)

71 Pytela R et al., Identification and isolation of a 140 kd cell surface glycoprotein with properties expected of a fibronectin receptor, Cell 40, 191–198 (1985)

72 Rosenblum BB et al., Two-dimensional electrophoretic analysis of erythrocyte membranes, Clin Chem 28, 925–931 (1982)

73 Simonds WF et al., Purification of the opiate receptor of NG108-15 neuroblastoma-glioma hybrid cells, Proc Natl Acad Sci USA 82, 4974–4978 (1985)

74 Sparrow LG et al., Purification and partial amino acid sequence of asialo murine granulocyte-macrophage colony stimulating factor, Proc Natl Acad Sci USA 82, 292–296 (1985)

75 Sprecher DL et al., Two-dimensional electrophoresis of human plasma apolipoprotein, Clin Chem 30, 2084–2092 (1984)

76 Stefano JE, Purified lupus antigen La recognizes an oligouridylate stretch common to the 3' termini of RNA polymerase III transcripts, Cell 36, 145–154 (1984)

77 Stephenson JR et al., Production and purification of murine monoclonal antibodies: aberrant elution from protein A-Sepharose 4B, Anal Biochem 142, 189–195 (1984)

78 Thudt K et al., Cloning and expression of the α-amylase gene from *Bacillus stearothermophilus* in several staphylococcal species, Gene 37, 163–169 (1985)

79 Toyama S and Toyama S, A variant form of β-actin in a mutant of KB cells resistant to cytochalasin B, Cell 37, 609–614 (1984)

80 Tsang VC et al., Enzyme-linked immunoelectrotransfer blot techniques (EITB) for studying the specificities of antigens and antibodies separated by gel electrophoresis, Methods Enzymol 92, 377–391 (1983)

81 Van Keuren ML et al., Detection of radioactively labeled proteins is quenched by silver staining methods: quenching is minimal for ^{14}C and partially reversible for ^{3}H with a photochemical stain, Anal. Biochem 116, 248–255 (1981)

82 Van Zoelen EJ et al., Neuroblastoma cells express *c-sis* and produce a transforming growth factor antigenically related to the platelet-derived growth factor, Mol Cell Biol 5, 2289–2297 (1985)

83 Vauhkonen M et al., Solubilization of proteins in dimethyl sulfoxide by permethylation: application to structural studies of apolipoprotein B, Anal Biochem 148, 357–364 (1985)

84 Wahl AF et al., Immunoaffinity purification and properties of a high molecular weight calf thymus DNA α-polymerase, Biochemistry 23, 1895–1899 (1984)

85 Welte K et al., Purification and biochemical characterization of human pluripotent hematopoietic colony-stimulating factor, Proc Natl Acad Sci USA 82, 1526–1530 (1985)

86 Yanagawa SI et al., Isolation of human erythropoietin with monoclonal antibodies, J Biol Chem 259, 2707–2710 (1984)

Ordering Information

Catalog #	Description
161-0443	Silver Stain Kit, includes oxidizer concentrate, silver reagent concentrate, developer, and instructions; enough to stain approximately 24 gels
161-0450	Silver Stain Developer, 115 g
161-0447	Silver Stain Developer, 4 x 115 g
161-0444	Oxidizer Concentrate, 480 ml
161-0445	Silver Reagent Concentrate, 480 ml
161-0436	Coomassie Brilliant Blue R-250 staining solution, 1 L
161-0400	Coomassie Brilliant Blue R-250, 10 g
161-0406	Coomassie Brilliant Blue G-250, 10 g

Related Products for SDS-PAGE and 2-D Electrophoresis

- Acrylamide powders, premixed powders, premixed solutions
- Crosslinkers and catalysts
- Ready Gel® precast gels
- Premixed buffer solutions
- Ampholytes
- Silver Stain Plus™ kit
- Copper stain kit
- Stains and tracking dyes
- Protein standards
- Criterion™ precast gel system
- ReadyStrip™ IPG strips
- ReadyPrep™ 2-D starter kit
- ReadyPrep sequential extraction kit

Related Products for DNA and Immunoelectrophoresis

- Agarose: standard grades, ultrapure grades, tablets
- Premixed immunoelectrophoresis buffers
- DNA standards
- Ethidium bromide

Other Related Kits from Bio-Rad

- Protein assay kits
- DNA purification kits

For information on any of these products, refer to the current Bio-Rad catalog or call your local Bio-Rad representative.

* Coomassie is a trademark of Imperial Chemical Industries PLC.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *(800) 4BIORAD* ***Australia*** *02 9914 2800* ***Austria*** *(01)-877 89 01* ***Belgium*** *09-385 55 11* ***Brazil*** *55 21 507 6191* ***Canada*** *(905) 712-2771* ***China*** *86-10-8201-1366/68* ***Denmark*** *45 44 52-1000* ***Finland*** *358 (0)9 804 2200* ***France*** *01 47 95 69 65* ***Germany*** *089 318 84-177* ***Hong Kong*** *852-2789-3300* ***India*** *(91-124) 6398112/113/114* ***Israel*** *03 951 4124* ***Italy*** *34 91 590 5200* ***Japan*** *03-5811-6270* ***Korea*** *82-2-3473-4460* ***Latin America*** *305-894-5950* ***Mexico*** *52 5 534 2552 to 54* ***The Netherlands*** *0318-540666* ***New Zealand*** *64-9-4152280* ***Norway*** *47-23-38-41-30* ***Russia*** *7 095 979 98 00* ***Singapore*** *65-2729877* ***Spain*** *34-91-590-5200* ***Sweden*** *46 (0)8-55 51 27 00* ***Switzerland*** *061-717-9555* ***United Kingdom*** *0800-181134*