

Serum Protein Electrophoresis Procedure

Helena  Laboratories

The Serum Protein Electrophoresis procedure is intended for the separation and quantitation of serum proteins using cellulose acetate electrophoresis.

SUMMARY

Serum contains over one hundred individual proteins, each with a specific set of functions and subject to specific variation in concentration under different pathologic conditions.¹ Since the introduction of moving-boundary electrophoresis by Tiselius² and the subsequent use of zone electrophoresis, serum proteins have been fractionated on the basis of their electrical charge into five classical fractions: albumin, alpha₁, alpha₂, beta, and gamma proteins. Each of these classical electrophoretic zones (with the exception of albumin) normally contains two or more components. Approximately fifteen serum proteins have been studied extensively because they may be measured easily.³⁻⁵

PRINCIPLE

Proteins are large molecules composed of covalently linked amino acids. Depending on electron distributions resulting from covalent or ionic bonding of structural subgroups, proteins have different electrical charges at a given pH. In the Helena Serum Protein procedure, the proteins are separated according to their respective electrical charges at pH 8.8 on a cellulose acetate plate using both the electrophoretic and electroosmotic forces present in the system. After the proteins are separated, the plate is placed in a solution of sulfosalicylic acid and Ponceau S (to stain the protein bands). The staining intensity is related to protein concentration.⁶ After dehydration in methanol, the plate background is then rendered transparent by treatment with a clearing solution.

REAGENTS

1. Ponceau S Stain (Cat. No. 5526)

Ingredients: After dissolution, each bottle of stain contains 0.5% (w/v) Ponceau S in an aqueous solution of 10% (w/v) Sulfosalicylic Acid.

WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST. HARMFUL IF SWALLOWED.

Preparation for Use: One vial of Ponceau S Stain is dissolved in 1 L of deionized water. Mix until thoroughly dissolved.

Storage and Stability: The stain may be stored as packaged or in a tightly closed staining dish at 15-30°C. The unopened stain is stable until the expiration date on the bottle.

Signs of Deterioration: The stain should be a homogeneous mixture free of precipitate. Do not use if excessive evaporation occurs or if large amounts of precipitate occur.

2. Electra® HR Buffer (Cat. No. 5805)

Ingredients: HR Buffer is a Tris-barbital-Sodium Barbital buffer.

WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST. The buffer contains barbital which, in sufficient quantity, can be toxic.

Preparation for Use: Dissolve one package of dry buffer in 750 mL of deionized water. The buffer is ready for use when all material is dissolved and completely mixed.

Storage and Stability: The packaged buffer is stable until the expiration date on the package. The diluted buffer is stable for 2 months at 15-30°C when stored tightly closed.

Signs of Deterioration: Discard packaged buffer if the material shows signs of dampness or discoloration. Discard unused diluted buffer if it becomes turbid.

3. Titan® III Cellulose Acetate Plate (Cat. No. 3013, 3023, 3024, 3033)

Ingredients: Cellulose acetate plates.

WARNING: FOR IN-VITRO DIAGNOSTIC USE

Preparation for Use: The plates are ready for use as packaged.

Storage and Stability: The plates should be stored at 15 to 30°C and are stable indefinitely.

4. Clear Aid (Cat. No. 5005)

Ingredients: Clear Aid contains polyethylene glycol.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.

Preparation for Use: When preparing the clearing solution, mix 30 parts glacial acetic acid, 70 parts absolute methanol, and 4 parts Clear Aid. Stir until well mixed.

Storage and Stability: The prepared Clear Aid should be stored in a tightly closed container at 15 to 30°C to prevent evaporation of the methanol. When evaporation occurs, the plates may delaminate. Water contamination, from over-use of the clearing solution, will cause the plate to be cloudy. The Clear Aid is stable until the expiration date on the bottle label.

Signs of Deterioration: Clear Aid should be a clear, colorless liquid, although it may appear cloudy when cold. Do not use the material upon evidence of gross contamination or discoloration. Discard the prepared Clear Aid solution if the plates appear cloudy after the clearing procedure.

5. PermaClear Solution (Cat. No. 4950)-Optional

Ingredients: N-methyl pyrrolidinone and PEG.

WARNING: FOR IN-VITRO DIAGNOSTIC USE - IRRITANT DO NOT PIPETTE BY MOUTH. VAPOR HARMFUL. In case of contact, flush affected areas with copious amounts of water. Get immediate attention for eyes.

Preparation for Use: Add 55 mL PermaClear to 45 mL deionized water to make working clearing solution. Mix well.

Storage and Stability: PermaClear should be stored at 15 to 30°C and is stable until the expiration date on the bottle.

Signs of Deterioration: Discard the PermaClear Solution if the plates turn white and do not clear as expected.

INSTRUMENTS

Any high quality scanning densitometer with visible transmittance capability may be used. Recommended is the Helena QuickScan 2000 or the EDC.

SPECIMEN COLLECTION AND HANDLING

Specimen: Fresh serum is the preferred specimen. The use of plasma should be avoided, as fibrinogen will appear as a distinct narrow band between the beta and gamma fractions. Cerebrospinal fluid may be used if concentrated approximately 100 times; urine may be used if concentrated up to 300 times, depending on original protein concentration.

Interfering Factors:

1. Hemolysis may cause false elevation in the alpha₂ and beta fractions.

2. Inaccurate results may be obtained on specimens left uncovered, due to evaporation.

Storage and Stability: If storage is necessary, samples may be stored covered at 2 to 8°C for 48 hours. Cerebrospinal fluid and urine specimens may be used after proper concentration with a concentrator.

PROCEDURE

Materials provided but not contained in a kit: The following materials are needed for the Protein Electrophoresis procedure. All items are available on an individual basis.

HARDWARE

	Cat. No.
Super Z Applicator	4084
Super Z Sample Well Plate (2)	4085
Super Z Aligning Base	4086
Super Z-12 Applicator	4090
Super Z-12 Sample Well Plate (2)	4096
Super CPK Aligning Base	4094
TITAN GEL Electrophoresis Chamber	4063
1000 Staining Set	5122
5 µL Microdispenser and Tubes	6008
Bufferizer	5093

CONSUMABLES

Zip Zone® Prep	5090
Titan Gel III Cellulose Acetate Plates	
25 x 76 mm	3013
60 x 76 mm	3023
76 x 76 mm	3033
94 x 76 mm	3024
Glue Stick	5002
Blotter Pads (76 x 102 mm)	5034
Titan Plastic Envelopes (63 x 120 mm)	5052
Blotter Pads (102 x 108 mm)	5037
Titan Plastic Envelopes (102 x 120 mm)	5053
Helena Marker	5000
Titan Identification Labels	5006
Ponceau S Stain	5526
Electra® HR Buffer	5805
Clear Aid	5005
PermaClear	4950
Electrophoresis Serum Control	5112
Zip Zone® Chamber Wicks	5081
Kemtrol Serum Control-Normal	7024
Kemtrol Serum Control-Abnormal	7025
Titan Plus Power Supply	1504

Materials needed, but not provided:

5% acetic acid (v/v): Add 50 mL of glacial acetic acid to 950 mL of deionized water.
Absolute methanol, reagent grade

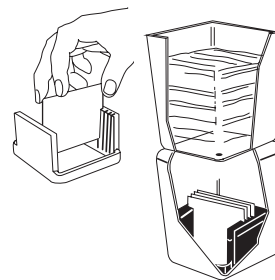
SUMMARY OF CONDITIONS

Plate	Titan III Cellulose Acetate Plate
Plate Soaking Time	20 minutes
Buffer Dilution	750 mL
Sample Volume	3 µL
Chamber Voltage	180 volts
Electrophoresis Time	15 minutes
Stain Time	6 minutes
Destain Time in 5% Acetic Acid	3 times/2 minutes each
Dehydration Time in Methanol	2 times/2 minutes each
Clearing Time (Clear Aid)	5-10 minutes
Drying Temperature	50-60°C
Drying Time	15 minutes
Scanning Wavelength	525 nm

STEP-BY-STEP METHOD

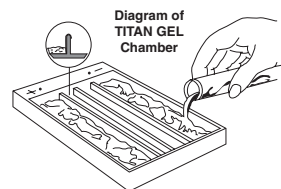
A. Titan III Plate Preparation

1. Properly code the required number of Titan III Plates by marking on the glossy, hard side with a Helena marker. It is suggested that the identification mark be placed in one corner so that it is always aligned with sample No. 1.
2. Soak the plates for 20 minutes in diluted Electra HR buffer. The plates should be soaked in the Bufferizer according to the instructions for use included with the Bufferizer. Alternately, the plates may be wetted by slowly and uniformly lowering a rack of plates into the HR Buffer such that air is not trapped in the plates. The same soaking buffer may be used for soaking up to 12 plates or for approximately one week if stored tightly closed. If used for a more prolonged period residual solvents from the plate may build up in the buffer or evaporation may alter buffer concentration.



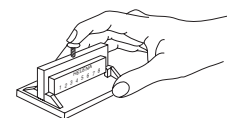
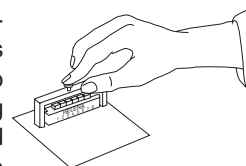
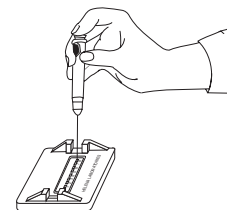
B. Electrophoresis Chamber Preparation

1. Pour approximately 100 mL of diluted HR Buffer into each of the outer sections of the electrophoresis chamber. Do not use the same buffer in which the plates were soaked for electrophoresis.
2. Wet two disposable wicks in the buffer. Stand them lengthwise (on edge) in the buffer compartments. Fold the top edge of each wick over each support bridge, making sure the bottom edge is in the buffer and touching the bottom of the chamber. Press the top edge down over the bridge until the wick makes contact with the buffer, and there are no air bubbles under the wicks.
3. Cover the chamber to saturate the air with buffer. Discard electrophoresis buffer after use.



C. Sample Application

1. Fill each well in the sample plate with 3 µL of sample using the microdispenser. Expel the samples as a bead on the tip of the glass tube; then touch this bead to the well. Cover the samples with a glass slide if they are not used within 2 minutes.
2. Prime the applicator by depressing the tips into the sample wells 3 or 4 times. Apply this loading to a piece of blotter paper. Priming the applicator makes the second loading much more uniform. Do not load applicator again at this point, but proceed quickly to the next step.
3. Remove the wetted Titan III Plate from the buffer with the fingertips and blot once firmly with a blotter. Before placing the plate in the aligning base, place a drop of water or buffer on the center of the aligning base. This prevents the plate from shifting during the sample application. Place



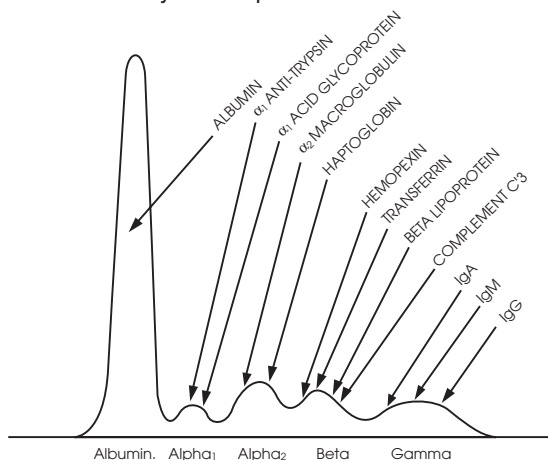
slightly increased alpha₁, and normal or increased gamma fractions (largely of maternal origin). The gamma drops rapidly until about 3 months of age, while the other fractions have reached adult levels by this time. Adult levels of the gamma globulins are not reached until 10-16 years of age. The albumin decreases and beta globulins increase after the age of 40.

Further Testing Required

The serum protein electropherogram or densitometric tracing should be evaluated for abnormalities. If abnormalities are observed, appropriate follow-up studies should be initiated. These may include immunoelectrophoresis, immunofixation, quantitation of individual component immunoglobulins, bone marrow examination, and other appropriate tests.

INTERPRETATION OF RESULTS^{5, 7}

Results on normal individuals will cover age and sex-related variations and day-to-day biologic variations. Disease states in which abnormal patterns are observed include inflammatory response, rheumatic disease, liver diseases, protein-loss disorders, plasma cell dyscrasias, and genetic deficiencies. Variant patterns have also been observed during pregnancy. Below is a normal serum protein pattern showing the locations of some of the more commonly known proteins.



SPECIFIC PERFORMANCE CHARACTERISTICS

Precision: A normal serum was run 26 consecutive times. The following data were obtained:

	Mean (Relative %)	S.D.	C.V.(%)
Albumin	55.7	1.4	2.5
Alpha ₁	3.1	0.4	12.5
Alpha ₂	11.3	0.4	3.8
Beta	11.8	0.5	4.0
Gamma	18.1	0.6	3.5

Linearity: Since the stain uptake is different for each band, the serum protein procedure is not linear. Do not dilute specimens which have a high total protein concentration. Use only 3 µL of specimen in the sample well plate.

BIBLIOGRAPHY

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SERUM PROTEIN KITS	
HARDWARE	Cat. No.
Super Z Applicator	4084
Super Z-8 Sample Well Plate (2)	4085
Super Z Aligning Base	4086
Super Z-12 Applicator	4090
Super Z-12 Sample Well Plate (2)	4096
Super CPK Aligning Base	4094
TITAN GEL Electrophoresis Chamber	4063
1000 Staining Set	5122
Microdispenser and Tubes	6008
Bufferizer	5093
CONSUMABLES	
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Electra HR Buffer	5805
Clear Aid	5005
PermaClear	4950
Electrophoresis Serum Control	5112
Zip Zone® Chamber Wicks	5081
Kemtrol Serum Control-Normal	7024
Kemtrol Serum Control-Abnormal	7025
Titan Plus Power Supply	1504

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