

Serum Protein Electrophoresis Procedure

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 Touch/2000.

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 |
| Zip Zone® Chamber Wicks | 5081 |
| SPE Normal Control | 3424 |
| SPE Abnormal Control | 3425 |
| Titan Plus Power Supply | 1504 |

Materials needed, but not provided:

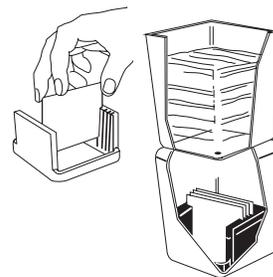
5% acetic acid (v/v): Add 50 mL of glacial acetic acid to 950 mL of deionized water.
 Absolute methanol, reagent grade

| 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

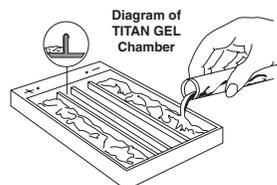
A. Titan III Plate Preparation

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.



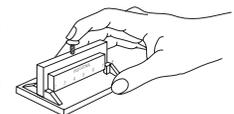
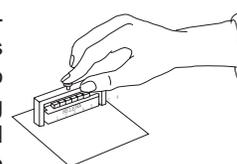
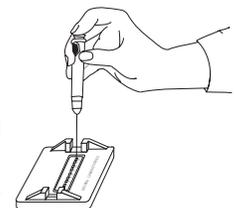
B. Electrophoresis Chamber Preparation

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

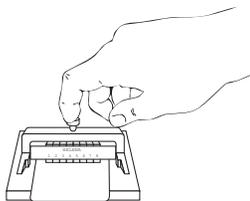
1. Fill each well in the sample plate with 3 µL of sample using the microdispenser. Expel the samples as a bead on the tip of the glass tube; then touch this bead to the well. Cover the samples with a glass slide if they are not used within 2 minutes.
2. Prime the applicator by depressing the tips into 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



the plate in the aligning base, cellulose acetate side up, aligning the bottom edge of the plate with the black scribe line marked "CENTER APPLICATION". The identification mark should be aligned with sample No. 1.

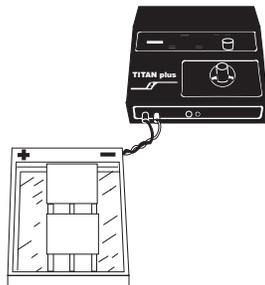


4. Apply the sample to the plate by gently depressing the applicator tips into the sample well 3 or 4 times and promptly transferring the applicator to the aligning base. Press the button down and hold it 5 seconds.



D. Electrophoresis

1. Quickly place the plate(s) cellulose acetate side down, in the electrophoresis chamber. Place a weight (glass slide, coin, etc.) on the plate(s) to insure contact with the wicks. Cover the chamber securely and wait 30 seconds for the plate(s) to equilibrate.



2. Electrophorese the plate(s) for 15 minutes at 180 volts. Power must be applied within 5 minutes after the plate(s) has been placed in the chamber.

E. Visualization of the Protein Bands

1. At the end of the electrophoresis time, remove the plate(s) from the chamber. Place them in 40-50 mL of Ponceau S stain (sufficient volume to cover the plate(s)) for 6 minutes. When staining 2 or more plates, carry out the protocol vertically in a rack. The stain may be reused until the plate background contains stain precipitate.
2. Destain in 3 successive 2 minute washes of 5% acetic acid or until the plate background is white. The plates may be dried and stored as a permanent record at this point if stored in a plastic envelope to protect the surface. If a transparent background is desired (i.e. for densitometry), proceed to the next step.

If using Clear Aid Solution:

3. Dehydrate by rinsing the plate in two absolute methanol washes for two minutes each wash. Allow the plate to drain for 5-10 seconds before placing in the next solution.
4. Place the plate into the clearing solution for 5-10 minutes.
5. Drain off excess solution. Then place the plate, acetate side up, onto a blotter, and into a drying oven at 50-60°C for 15 minutes or until dry.

If using PermaClear Solution:

3. Place the plate(s) into the diluted PermaClear clearing-solution for 2 minutes.
4. Drain off excess solution by holding plate(s) vertically for 1 minute. Then place the plate, acetate side up, onto a blotter, and into a drying oven at 50-60°C for 15 minutes or until dry.

F. Evaluation of the Protein Bands

Scan the plates on QuickScan using a slit size of 5.

Stability of End Product

The completed, dried serum protein plate is stable for an indefinite period of time and may be stored in Titan Plastic Envelopes.

Calibration

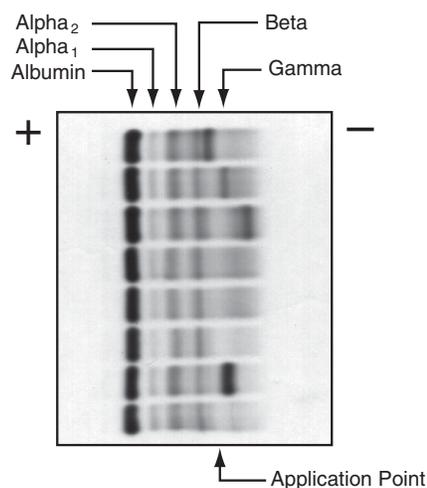
The Optical Density Step Tablet (Cat. No. 1047) should be used to insure the linearity of the instrument and a Neutral Density Densitometer Control (Cat. No. 1031) should be used to validate the zero adjustment and quantitation by the instrument.

QUALITY CONTROL

SPE Normal (Cat. No. 3424) and SPE Abnormal (Cat. No. 3425) Control may be used to verify all phases of the procedure and should be used on each run. Refer to the package insert provided with the control for assay values.

RESULTS

The fastest moving band, and normally the most prominent, is the albumin band found closest to the anodic edge of the plate. The faint band next to this is alpha₁ globulin, followed by alpha₂ globulin, beta, and gamma globulins. Prealbumin is seldom visible with this system.



Calculation of the Unknown

The QuickScan Touch/2000 will automatically print the relative percent and the absolute values for each band. Alternately, the relative percent of each band can be calculated manually by referring to the Operator's Manual provided with the densitometer. The relative percent of each band is calculated by the following formula:

$$\frac{\text{No. Integration Units of the Band}}{\text{Total Integration Units}} \times 100 = \text{Relative Percent of the Band}$$

$$\frac{\text{Relative Percent of the Band}}{100} \times \text{Total Serum Protein} = \text{Absolute Value of Protein per band}$$

REFERENCE VALUES

The reference values for serum protein electrophoresis on cellulose acetate stained with Ponceau S were determined from a study of 51 normal subjects. These values are for illustrative purposes only. Each laboratory should establish its own range.

| Protein Fraction | Concentration |
|--------------------|------------------|
| Albumin | 3.63 - 4.91 g/dL |
| Alpha ₁ | 0.11 - 0.35 |
| Alpha ₂ | 0.65 - 1.17 |
| Beta | 0.74 - 1.26 |
| Gamma | 0.58 - 1.74 |

Variations of Expected Values⁴

Studies show that values are the same for both males and nonpregnant females. (Some differences are seen in pregnant females at term and in women on oral contraceptives.) Age has some effect on normal levels. Cord blood has decreased total protein, albumin, alpha₂ and beta fractions, slightly increased alpha₁, and normal or increased gamma fractions (largely of

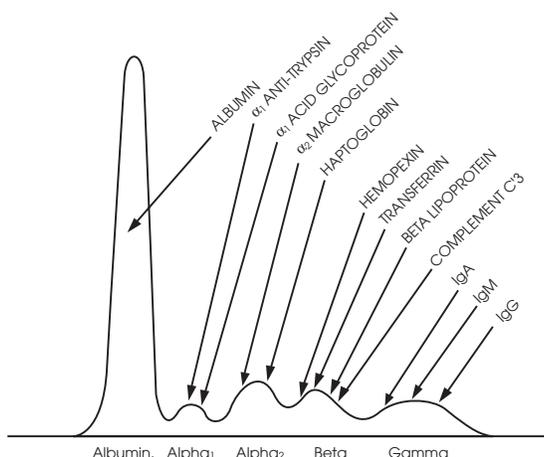
maternal origin). The gamma drops rapidly until about 3 months of age, while the other fractions have reached adult levels by this time. Adult levels of the gamma globulins are not reached until 10-16 years of age. The albumin decreases and beta globulins increase after the age of 40.

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.

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