preliminarily increased alpha, and normal or increased gamma frac-
tions (largely of maternal origin). The gamma crosses 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 electrophoresis or densitometric tracing
should be evaluated for abnormalities. If abnormalities are
observed, appropriate follow-up studies should be initiated.
These may include immuneelectrophoresis, immunofixation,
quantitation of individual component immunoglobulins, bone
marrow examination, and other appropriate tests.
INTERPRETATION OF RESULTS
Results on normal individuals will cover age and sex-related
variations and day-to-day biologic variations. Disease states
in which abnormal proteins are observed include inflammatory
response, rheumatic disease, liver diseases, protein-losing disor-
ders, 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:

<table>
<thead>
<tr>
<th>SPECIFIC</th>
<th>PERFORMANCE</th>
<th>CHARACTERISTICS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

PRINCIPLE
Proteins are large molecules composed of covalently linked
amino acids. Depending on electron distributions resulting from
covalent or ionic bonding of structural subgroups, pro-
tiens 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 plate using both the electrophoretic and elec-
troosmotic 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. After dehydration
in methanol, the plate background is then rendered transparent
by treatment with a clearing solution.

REAGENTS
1. Ponceau S Stain (Cat. No. S5526)
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.
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
vial is stable for at least 1 year.

2. Electrol HRF Buffer (Cat. No. S5805)
Ingredients: A clear solution of Tris-barbital-Sodium barbituric buffer.

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

The Serum Protein Electrophoresis procedure is intended for
the identification and quantitation of serum proteins using cellu-
lose acetate electrophoresis.

SERUM PROTEIN KITS

SERUM PROTEIN KITS

HARDWARE
Cat. No.

Super Z Applicator

Super Z Sample Well Plate (2)

Super Z Aligning Base

Super Z 12-Applicator

Super Z 12-Sample Well Plate (2)

Super CPK Aligning Base

TITAN GEL Electrophoresis Chamber

1000 Staining Set

Microdispenser and Tubes

Bufferzer

CONSUMABLES
Zip Zone® Prep

Titan Gel III Cellulose Acetate Plates

5 x 76 mm

25 x 76 mm

76 x 76 mm

Titan Plastic Envelopes (63 x 120 mm)

Titan Plastic Envelopes (102 x 120 mm)

Helena Marker

Titan Identification Labels

Ponceau S

Electra HRF Buffer

Clear Aid

PermaClear

Electrophoresis Serum Control

Zip Zone® Chamber Wicks

Kemtrol Serum Control-Non

Kemtrol Serum Control-Abnormal

Titan Plus Power Supply

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In no case will Helena Laboratories be liable for consequential damages even if Helena has been advised as to the possibility of such damage.

Still areas indicate that the text has been modified, added or deleted.

<table>
<thead>
<tr>
<th>SPECIFICATIONS</th>
<th>PERFORMANCE</th>
<th>CHARACTERISTICS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES
1. Alper, C.A., Plasma Protein Measurements as a Diagnostic Aid. N
2. Tiselius, A., New Approach for Electrophoretic Analysis of Colloidal
3. Ritzenmann, S.E. and Daniels, J.C., Diagnostic Proteinology,
Separation and Characterization of Proteins. Qualitative and Quantitative
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**
- 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: 4083
- 1000 Staining Set: 5122
- 5 µL Microdispenser and Tubes: 6008
- Zip Zone: 5093
- Buffer: 5092

**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 (120 mm): 5025
- Titan Plastic Envelopes (102 x 108 mm): 5037
- Helena Marker: 5000
- Titan Identification Labels: 5006
- Titan III Stain: 5530
- Electra® HR Buffer: 5805
- Clear Aid: 5005
- Perm Clear: 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

**SUMMARY OF CONDITIONS**

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

**STEP-BY-STEP METHOD**

### A. Titan III Plate Preparation

1. Pour an approximate 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 buffer according to the instructions for use included with the Buffer. Alternately, the plates may be wetted by slowly and uniformly lowering a rack of plates into the buffer. The same soaking buffer may be used for soaking up to 15 minutes 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.

### 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 and 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. Do not make a vacuum seal.

### C. Sample Application

1. Fill each well in the sample plate with 3 µL of sample using the Microdispenser and Tubes provided. Expose 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 applicator 3 or 4 times. Apply this loading to the electrophoresis chamber.

### D. Electrophoresis

1. Place the plate(s) into the diluted PermaClear clearing solution. Place a weight 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) have 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 buffer with the fingertips and blot them once firmly with a blotter, and into a drying oven at 50-60°C for 15 minutes or until dry.

2. Drain off excess solution. Then place the plate(s) in two absolute methanol washes for 2 minutes each. Wash the plate(s) 2 or more plates, carry out the protocol vertically in a rack. The stain may be reused and held it 5 seconds.

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

1. Scan the plate(s) in a densitometer using a 525 nm filter and the narrow slit (size 4).

2. Calibration of the Optical Density Step Tablet (Cat. No. 1047) should be used to insuring the Optical Density and a Neutral Density Densitometer Control (Cat. No. 1032) should be used to validate the zero adjustment and quantitation by the instrument.

### QUALITY CONTROL

Electrophoresis Serum Control (Cat. No. 5112), Kemtrol Serum Control-Normal (Cat. No. 7024) or Kemtrol Serum Control-Abnormal (Cat. No. 7025) 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 anodal edge of the gel. The faint band next to this is alpha, globulin, followed by alpha, globulin, beta, and gamma globulins. Prewidenin is seldom visible with this system.

### Calculation of the Unknown

The Helena densitometers with computer accessories 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 absolute percent of each band is calculated by the following formula:

\[ \text{Relative Percent} = \frac{\text{Total Integration Units the Band}}{\text{Total Integration Units the Band}} \times 100 \]

### 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.

### Variations of Expected Values

Studies show that values are the same for both males and nonpregnant females. (Some differences are seen in pregnant female at term and in women on oral contraceptives.) Age has some effect on normal levels. Cord blood has decreased total protein, albumin, alpha, 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 centrifuge.

PROCEEDURE

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**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>4084</td>
<td>Super Z Applicator</td>
</tr>
<tr>
<td>4085</td>
<td>Super Z Sample Well Plate (2)</td>
</tr>
<tr>
<td>4086</td>
<td>Super Z Aligning Base</td>
</tr>
<tr>
<td>4089</td>
<td>Super Z-12 Applicator</td>
</tr>
<tr>
<td>4096</td>
<td>Super Z-12 Sample Well Plate (2)</td>
</tr>
<tr>
<td>4096</td>
<td>Super CPK Aligning Base</td>
</tr>
<tr>
<td>4098</td>
<td>TITAN GEL Electrophoresis Chamber</td>
</tr>
<tr>
<td>5122</td>
<td>1000 Staining Set</td>
</tr>
<tr>
<td>6008</td>
<td>5 µL Microdispenser and Tubes</td>
</tr>
<tr>
<td>5033</td>
<td>Bufferzer</td>
</tr>
</tbody>
</table>

**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 |
| Blotters Pads (76 x 102 mm) | 5005 |
| Titanium Envelopes (120 x 120 mm) | 5002 |
| Blotters Pads (102 x 108 mm) | 5037 |
| Titan Plastic Envelopes (120 x 120 mm) | 5053 |
| Helena Marker | 5000 |
| Titan Identification Labels | 5006 |
| Titan Stain | 5520 |
| Electra® HR Buffer | 5005 |
| Clear Aid | 5000 |
| PrintClear | 5000 |
| Electrophoresis Serum Control | 5112 |
| Zip Zone™ Chamber Wicks | 5081 |
| Kemtral Serum Control Normal | 7024 |
| Kemtral 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**

<table>
<thead>
<tr>
<th>Plate</th>
<th>10 minutes</th>
<th>Time in Acetic Acid 10 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate Soaking Time</td>
<td>20 minutes</td>
<td>Buffer Dilution: 750 mL</td>
</tr>
<tr>
<td>Sample Volume</td>
<td>3 mL</td>
<td>Chamber Voltage: 180 volts</td>
</tr>
<tr>
<td>Electrophoresis Time</td>
<td>15 minutes</td>
<td>Stain Time: 6 minutes</td>
</tr>
<tr>
<td>Destain Time 5% Acetic Acid 3 times/2 minutes</td>
<td></td>
<td>Dehydration Time in Methanol: 2 times/2 minutes</td>
</tr>
<tr>
<td>Clearing Time (Clear Aid)</td>
<td>5-10 minutes</td>
<td>Clearing Time (Clear Aid)</td>
</tr>
</tbody>
</table>

**STEP-BY-STEP METHOD**

**A. Titan III Plate Preparation**

1. Pour into 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. Pour the plates for 20 minutes in diluted Electro HR buffer. The plates shall 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 Titan III Cellulose Acetate Plates.

**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 the edge) in the buffer compartments. Fold the top edge of each wick and 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.

**C. Sample Application**

1. Fill each well in the sample plate with 5 µL of cell lysate or 37 µL of serum by using the microdispenser. Expose 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 load onto 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 blackscribe line marked “APPLICATION”. The identification mark should be aligned with sample No. 1.

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

**D. Electrophoresis**

1. Quickly place the plates (cellulose acetate side down) into 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.

3. Dehydrate by rinsing the plate in two absolute methanol 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.

**E. Visualization of the Protein Bands**

1. Place the plate(s) into a drying oven at 50-60°C for 15 minutes or until dry.

2. Scrape Ponceau S stain (sufficient volume to cover the plate(s) for 15 minutes) and a small amount of water (e.g. 100µL) onto a blotter, and into a drying oven at 50-60°C for 15 minutes or until dry.

3. Using Clear Aid Solution:

   a. Dehydrate by rinsing the plate in two absolute methanol washes for two minutes each. Wash the plate to remove excess 5% acetic acid or more plates, carry out this protocol vertically in a rack. The stain may be reused until the plate background contains stain precipitate.

   b. 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.

**F. Evaluation of the Protein Bands**

1. Scan the plates in a densitometer using a 525 nm filter and Ponceau S stain (sufficient volume to cover the plate(s) for 15 minutes) and a small amount of water (e.g. 100µL) onto a blotter, and into a drying oven at 50-60°C for 15 minutes or until dry.

2. Using PemClear Solution:

   a. Place the plate(s) into the diluted PemClear clearing solution for 2 minutes.

   b. Drain off excess solution by holding plate(s) vertically for 1 minute. Then place the plate, acetate side down, onto a blotter, and into a drying oven at 50-60°C for 15 minutes or until dry.

3. F. Evaluation of the Protein Bands

   a. Scan the plates in a densitometer using a 525 nm filter and a narrow slit (size 4).

   b. Staining of End Product

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

**Calculation of the Unknown**

The Helena densitometers with computer accessories 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:

\[
\text{Relative Percent} = \frac{\text{Total Integration Units the Band}}{\text{Total Integration Units the Band}} 
\]

**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.

<table>
<thead>
<tr>
<th>Protein Fraction</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>3.63 - 4.91 g/dL</td>
</tr>
<tr>
<td>Alpha</td>
<td>0.11 - 0.35 g/dL</td>
</tr>
<tr>
<td>Beta</td>
<td>0.65 - 1.17 (\mu)g/mL</td>
</tr>
<tr>
<td>Gamma</td>
<td>1.74 - 2.62 g/dL</td>
</tr>
</tbody>
</table>

**Variations of Expected Values**

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, alpha, and beta fractions.
Slightly increased alpha-1 and normal or increased gamma fractions (largely of maternal origin). The gamma drops rapidly until about 3 months of age, while the alpha 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

Results on normal individuals will cover age and sex-related variations and day-to-day biologic variations. Disease states in which abnormal results are observed include inflammatory response, rheumatic disease, liver diseases, protein-losing 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:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>55.7</td>
<td>1.4</td>
<td>2.5</td>
</tr>
<tr>
<td>Alpha 1</td>
<td>3.1</td>
<td>0.4</td>
<td>12.5</td>
</tr>
<tr>
<td>Alpha 2</td>
<td>11.3</td>
<td>0.4</td>
<td>3.8</td>
</tr>
<tr>
<td>Beta 1</td>
<td>5.0</td>
<td>0.4</td>
<td>8.0</td>
</tr>
<tr>
<td>Gamma 1</td>
<td>18.1</td>
<td>0.6</td>
<td>3.5</td>
</tr>
</tbody>
</table>

**Linearity:** Since the stain uptake is different for each band, the serum protein procedure is not linear. Do not dilute specimens in methanol, the plate background is then rendered transparent for 2 months at 15-30°C when stored tightly closed.

**INGREDIENTS:**

- HR Buffer is a Tris-barbital-Sodium Barbital buffer.
- Sulfosalicylic Acid.
- 0.5% (w/v) Ponceau S in an aqueous solution of 10% (w/v) N-methyl pyrrolidinone and PEG.
- 48.5% Methanol.
- 100 times; urine may be used if concentrated up to 300 times, depending on original protein concentration.
- Tris-barital-Sodium Barbitol buffer.

**WARNING:** FOR IN-VITRO DIAGNOSTIC USE.

**LIMITATIONS:**

- Do not pipette by mouth.
- Vapors 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:**

- The plates are ready for use as pack-

**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. Since the introduction of moving-boundary electrophoresis by Tiselius\(^\text{1}\) and the subsequent use of zone electrophoresis, serum proteins have been fractionated on the basis of their electrical charge into five classical fractions: albumin, alpha acid, beta, alpha, 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.\(^\text{2-3}\)

**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 electroendosmosic 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. 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)
2. Electra RR Buffer (Cat. No. 5805)
3. Ponceau S
4. Clear Aid (Cat. No. 5005)
5. PermaClear
6. Sulfosalicylic Acid

**WARNING:** FOR IN-VITRO DIAGNOSTIC USE.

**PREPARATION FOR USE:**

- Dissolve 1.0 g of Ponceau S in 750 mL of deionized water. The buffer is ready for use when dissolved.

**STORAGE AND STABILITY:**

- The plates are ready for use as packed.

**SUMMARY**

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

**BIBLIOGRAPHY**

3. Ritzenmann, S.E. and Daniels, J.C., Diagnostic Proteinology, Separation and Characterization of Proteins, Qualitative and Quantitative Assays in Laboratory Medicine, Harper and Row Inc., Hagerstown, 1979.