TITAN GEL Serum Protein System

Cat. No. 3041

Helena Laboratories

The Helena TITAN GEL Serum Protein System is intended for the separation and quantitation of serum proteins by agarose gel 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 Tiselius[2] and the subsequent use of zone electrophoresis, serum proteins have been fractionated on the basis of their electrical charge at a particular pH into five classical fractions: albumin, alpha$_1$, alpha$_2$, beta and gamma proteins. Each of these classical electrophoretic zones 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 or structural subgroups, proteins can be either polar or nonpolar at a given pH. In the TITAN GEL Serum Protein procedure, proteins are separated according to their respective electrical charges at 8.4-8.8 on agarose gel using both the electrophoretic and electroendosmotic forces present in the system. The proteins are then stained with Amido Black staining solution.

REAGENT

1. **TITAN GEL Serum Protein Gel**

   Ingredients: Each gel contains agarose in barbital buffer with 0.01% thimerosal added as a preservative.

   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY.

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

   Storage and Stability: The gels should be stored at room temperature (15 to 30°C) and are stable until the expiration date indicated on the package. The gels must be stored in the protective packaging in which they are shipped. DO NOT REFRIGERATE OR FREEZE THE GELS.

   Signs of Deterioration: Any of the following conditions may indicate deterioration of the gel: (1) crystalline appearance indicating the agarose has been frozen, (2) cracking and peeling indicating drying of the agarose, (3) bacterial growth indicating contamination.

2. **TITAN GEL Serum Protein Buffer**

   Ingredients: The buffer is a barbital-sodium barbital buffer with 0.1% sodium azide added as a preservative; pH 8.4-8.8.

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

   The buffer contains barbital which, in sufficient quantity, can be toxic. To prevent the formation of toxic vapors, sodium azide should not be mixed with acidic solutions.When discarding reagents containing sodium azide, always flush sink with copious quantities of water. This will prevent the formation of metallic azides which, when highly concentrated in metal, are potentially explosive. In addition to purging with water, plumbing should occasionally be decontaminated with 10% NaOH.

   Preparation for Use: Dissolve one bag in 1500 mL of deionized water. The buffer is ready for use when all material is completely dissolved.

   Storage and Stability: The packaged buffer should be stored at 15 to 30°C and is stable until the expiration date indicated on the package. Diluted buffer is stable two months at 15 to 30°C.

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

3. **Amido Black Protein Stain**

   Ingredients: When reconstituted as directed, the stain contains 0.25% (w/v) Amido Black stain.

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

   Preparation for Use: Dissolve the dry stain (entire contents of vial) in 1 L of the Fixative/Destain Solution made in the “Materials needed but not provided” section. Mix thoroughly for 30 minutes.

   Storage and Stability: The dry stain should be stored at 15 to 30°C and is stable until the expiration date indicated on the package. The diluted stain is stable one year stored at 15 to 30°C.

   Signs of Deterioration: The diluted stain should be a homogeneous mixture free of precipitate. Discard if precipitate forms.

INSTRUMENTS

Any high quality scanning densitometer with visible transmittance capability may be used to scan the gels. Recommended is the Helena EDC® (Cat. No. 1376), the CliniScan™ 2 (Cat. No. 1260) or the CliniScan 3 (Cat. No. 1680). Refer to the Operator’s Manual for detailed instructions.

SPECIMEN COLLECTION AND HANDLING

Specimen: The specimen may be serum, plasma, urine or cerebrospinal fluid. Use of plasma will cause a fibrinogen band to appear as a distinct narrow band between the beta and gamma fractions.

Interfering Factors:

1. Hemolysis may cause false elevation in the alpha$_2$ and beta fractions.

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

Storage and Stability: Fresh serum or plasma is the specimen of choice. If storage is necessary, samples may be stored covered at 15 to 30°C for 4 days or 2 to 6°C for 2 weeks, or -20°C for 6 months.[7] Cerebrospinal fluid and urine specimens may be used after proper concentration (10-50X) with a concentrator.

PROCEDURE

Materials provided: The following materials needed for the procedure are contained in the TITAN GEL Serum Protein Kit (Cat. No. 3041). Individual items are not available.

TITAN GEL Serum Protein Gels (10)
TITAN GEL Serum Protein Buffer (1 pkg)
Amido Black Protein Stain (1 vial)
TITAN GEL Blotter A (20)
TITAN GEL SPE Templates (10)

Materials provided by Helena Laboratories but not contained in the kit:

ITEM	CAT. NO.
Dialamatic Microdispenser and Tubes	6210
SPE Control	5136
TITAN GEL Chamber	4063
I.O.D. (Incubator, Oven, Dryer)	5116
Titan Plus Power Supply	1504
TITAN GEL Multi-Staining Set	1558
Titan Blotter Pads	5037

Materials needed but not provided:

Glacial Acetic Acid
Methanol
Fixative/Destain Solution: Mix 1 L methanol, 1 L deionized water and 200 mL glacial acetic acid. Mix well. Use 1 L of this solution to prepare the stain solution and the remainder for destaining the gels.

SUMMARY OF CONDITIONS

Gel	TITAN GEL Serum Protein Gel
Buffer Dilution	1500 mL
Sample Dilution	1 part sample + 3 parts buffer
Sample Volume	3 µL
Serum Absorption Time	4 minutes
Electrophoresis Time	15 minutes
Voltage	120 V
Drying Time	5 minutes
Staining Time	10 minutes
Destaining Time	2 x 1 minute
Drying time (after destaining)	5 minutes
Scanning Wavelength	595 nm

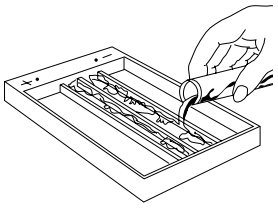
Recommended EWC Parameters:

Buffer volume; mL per chamber section	20 mL
Electrophoresis Voltage	85 V
Electrophoresis Time	20 minutes
Staining Time	10 minutes
Incubation Time/Temp	N/A
Drying Time	15 minutes
Drying Temperature	55°C

STEP-BY-STEP METHOD

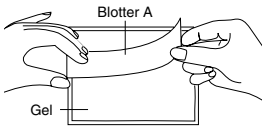
A. Preparation of the TITAN GEL CHAMBER

1. Dissolve one bag of TITAN GEL Serum Protein Buffer in 1500 mL of deionized water.
2. Pour approximately 25 mL of diluted buffer into each inner section of the chamber.
3. Cover the chamber until ready to use.

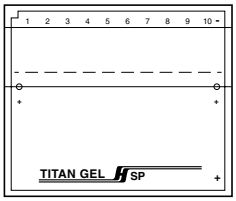


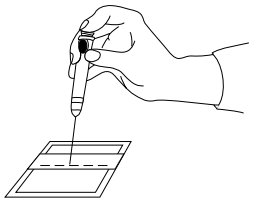
B. Sample Application

1. Dilute each patient sample and control 1:4 (1 part sample + 3 parts buffer) with TITAN GEL Serum Protein Buffer.
2. Remove the TITAN GEL Serum Protein Gel from the protective packaging. One edge of the agarose gel has been numbered for easy sample placement and identification.
3. Using Blotter A, gently blot the application area of the gel using a slight fingertip pressure on the blotter.

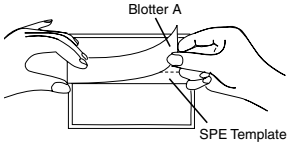


4. Carefully place the TITAN GEL SPE Template on the gel, aligning the application slits with the zero signs (0) on the sides of the gel and trying to avoid trapping any air bubbles under the template. Place a Blotter A over the template and remove any bubbles in the slit area with slight fingertip pressure. Retain the blotter for use in Step 7.





5. Place 3.0 µL of each sample onto the template slits, spreading the sample completely over the entire slit. Apply the samples as quickly as possible.
6. Wait 4 minutes after the last sample has been applied to allow the samples to diffuse into the agarose.
7. Gently blot the template with the Blotter A retained in Step 4 and then carefully remove the blotter.



8. Wait 30 seconds and then carefully remove the template.

C. Electrophoresis of the Sample Gel

1. Quickly place the gel into the inner section of the chamber, agarose side down, by gently squeezing the gel into place. Position the gel so that the edges of the agarose are in the buffer and the application

point is on the cathodic (-) side. Two gels may be electrophoresed at one time.

2. Place the cover on the chamber and insure that the cover does not touch the gel. Electrophorese the gel(s) at 120 volts for 15 minutes.

D. Visualization of the Protein Bands

1. At the end of the electrophoresis period, remove the gel from the chamber and place it in methanol for 5 minutes.
2. Remove the gel from the methanol and lay it on a blotter. Then place it into an I.O.D., or other laboratory drying oven with forced air at 60-70°C for 5 minutes or until dry. The gel may be dried at a lower temperature but additional time will be required. The gel will not destain properly if it is not completely dry.
3. Fill one container of the Staining Set with prepared stain. Fill another container with Fixative/Destain Solution.
4. Remove the gel from the oven and place it in the Staining Rack. Immerse the rack into the stain for 10 minutes.
5. Remove the rack from the stain and allow it to drain on a blotter. Destain the gel by rinsing it in two (2) consecutive washes of destain solution. Allow the gel to remain in each wash for 1 minute. The gel background should be completely clear. If the gel background is not completely clear, a final water wash should be used to remove trace amounts of stain. Place the gel in tap water for 1 minute after destaining it. Wipe the back of the gel with laboratory tissue dampened with methanol to remove any remaining stain.
6. Dry the destained gel by placing it on a blotter and into an I.O.D., or other drying oven at 60-70°C until dry.

E. Evaluation of the Protein Band

Scan the dried TITAN GEL Serum Protein Gel at 595 nm.

Stability of End Product

The completed, dried TITAN GEL Serumm Protein Gel is stable for a indefinite period of time.

Quality Control

SPE Control (Cat. No. 5136) may be used to verify all phases of the procedure and should be used on each gel run. Refer to the package insert provided with the control for assay values.

RESULTS

Figure 1 illustrates the electrophoretic mobilities of the albumin, alpha$_1$, alpha$_2$, beta and gamma protein bands on TITAN GEL Serum Protein Gel. The fastest moving band, and normally the most prominent, is the albumin band found closest to the anodic edge of the gel. The faint band next to this is alpha$_1$, globulin, followed by alpha$_2$ globulin, beta and gamma globulins.

Figure 2 illustrates a typical densitometric tracing produced with the Helena EDC. The protein bands are labeled as they appear in a normal protein pattern.

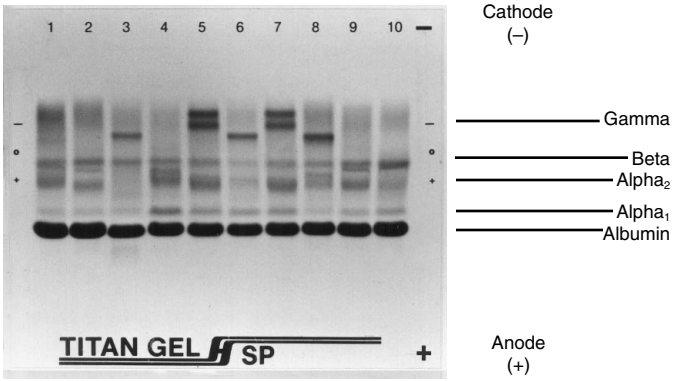


Figure 1: TITAN GEL Serum Protein Gel illustrating the electrophoretic mobilities of albumin and alpha$_1$, alpha$_2$, beta and gamma globulins.

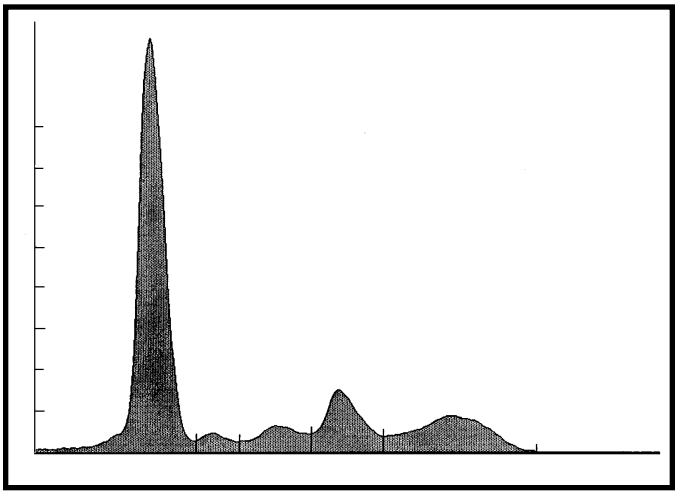


Figure 2: Densitometric tracing of serum protein electrophoresis pattern.

Calculation of the Unknown:

The Helena EDC Densitometer and other Helena densitometers with computer accessories will automatically print the relative percent and the absolute values for each band. Refer to the Operator's Manual provided with the densitometer.

REFERENCE VALUES

The reference values for serum protein electrophoresis on the TITAN GEL Serum Protein System are presented. These values are presented as a guideline. Each laboratory should establish its own normal range study.

Protein Fraction	% of Total Protein
Albumin	52.3 - 66.0
Alpha$_1$	3.3 - 7.0
Alpha$_2$	6.3 - 11.7
Beta	7.8 - 14.3
Gamma	11.1 - 20.4

Variations of Expected Values[4]

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$_2$ and beta fractions; slightly increased alpha$_1$ and normal or increased gamma fractions (largely of maternal origin). The gamma globulins drop rapidly until about three

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 globulin increases 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 immunoglobulins, bone marrow examination and other appropriate tests.

INTERPRETATION OF RESULTS[5, 6]

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, monoclonal gammopathies, pregnancy and genetic deficiencies.

LIMITATIONS

Since all electrophoretic procedures are nonlinear, it is critical to use the recommended volume of undiluted serum to obtain optimal resolution and reproducible results. Noncompliance with the recommended procedure may affect the results.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision: Within-Run and Run-to-Run precision studies yielded CV's of less than 10%.

Sensitivity: The sensitivity of the system, using the Amido Black Protein Stain, is 10 µg/dL.

Comparison: A comparison study of this method to the cellulose acetate method, using a range of 4.48 g/dL-11.85 g/dL, was excellent yielding a linear regression equation of Y = 0.996X + 0.072 (where X is the TITAN GEL method and Y is the cellulose acetate method) and a correlation coefficient of 0.998.

BIBLIOGRAPHY

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TITAN GEL Serum Protein Buffer (1 pkg.)
Amido Black Protein Stain (1 vial)
TITAN GEL Blotter A (20)
TITAN GEL SPE Templates (10)

Other Supplies and Equipment

The following items, needed for performance of the TITAN GEL Serum Protein Procedure, must be ordered individually.

	Cat. No.
Dialamatic Microdispenser and Tubes	6210
TITAN GEL Chamber	4063
I.O.D. (Incubation and Drying Oven)	5116
TITAN GEL Multi-Staining Set	1558
SPE Control (1 x 2 mL)	5136
Titan Plus Power Supply	1504
Titan Blotter Pads	5037

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