TITAN GEL High Resolution Protein System

Cat. No. 3040

The Helena TITAN GEL High Resolution Protein method is intended for the separation of protein fractions by agarose gel electrophoresis.

SUMMARY

High resolution electrophoresis achieves better resolution of the proteins beyond the classical five band patterns thereby increasing the diagnostic usefulness of protein patterns.[1-3] Approximately fifteen serum proteins have been studied extensively because they may be measured easily.[4-7] In this context, high resolution electrophoresis refers to systems which separate 95% of the total protein mass into 10-15 discrete fractions.

PRINCIPLE

Proteins are large molecules composed of covalently linked amino acids. Proteins can be either polar or nonpolar at a given pH depending on electron distributions resulting from covalent or ionic bonding of structural subgroups. In the Helena procedure, proteins are separated according to their respective electrical charges on agarose gel using both the electrophoretic and electroendosmotic forces present in the system. The separations are stained with a protein sensitive stain.

REAGENT

1. **TITAN GEL High Resolution Protein Gel**
   Ingredients: Each gel contains agarose in a barbital buffer with thimerosal added as preservatives.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY.
   CAUTION: The buffer contains barbital which, in sufficient quantity, can be toxic.
   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 High Resolution Protein Buffer**
   Ingredients: The buffer contains barbital, sodium barbital, calcium lactate and 0.1% sodium azide as a preservative.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.
   CAUTION: 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 plumbing, are potentially explosive. In addition to purging pipes with water, plumbing should occasionally be decontaminated with 10% NaOH.
   Preparation for Use: Dissolve one package of buffer in 1500 mL 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. The buffer solution is stable six months stored 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. **TITAN GEL High Resolution Protein Stain**
   Ingredients: The stain contains Coomassie Brilliant Blue.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.
   Preparation for Use: Dissolve the dry stain in 500 mL of methanol. Add 500 mL of purified water and acidify with 100 mL glacial acetic acid. Filter before use if necessary. Total volume: 1100 mL.
   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 for two months when stored at 15 to 30°C. Used stain may be returned to the bottle and re-used approximately two months.
   Signs of Deterioration: The diluted stain should be a homogeneous mixture free of precipitate.
4. **Amido Black Protein Stain (Cat. No. 3048, Optional stain)**
   Ingredients: The stain contains Amido Black.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE.
   Preparation for Use: Dissolve the contents of one vial of stain in 1 L Fixative/Destain Solution. Refer to "Materials Needed but not Supplied" section.
   Storage and Stability: The stain should be stored at 15 to 30°C and is stable until the expiration date indicated on the vial.
   Signs of Deterioration: The stain should be a homogeneous mixture free of precipitate.

SPECIMEN COLLECTION AND HANDLING

Specimen: The specimen may be serum, plasma, urine or cerebrospinal fluid. A fibrinogen band, which may obscure the beta-gamma zone, will appear in plasma samples.

Specimen Preparation:

Serum/Plasma: When staining with TITAN GEL High Resolution Protein Stain, dilute serum and plasma samples 1:2 with 0.85% saline (1 part serum or plasma and 1 part saline). However, when staining with Amido Black Protein Stain, apply serum and plasma samples undiluted.

Urine: Concentrate urine samples 100X with a concentrator.

CSF: Concentrate CSF samples 80-100X with a concentrator.

Storage Fresh serum or plasma is the specimen of choice. If storage is necessary, samples may be stored covered at 2 to 8°C for 48 hours.

PROCEDURE

Materials provided: The following materials needed for the procedure are contained in the TITAN GEL High Resolution Protein Kit (Cat. No. 3040). Individual items are not available.

- TITAN GEL High Resolution Protein Gels (10)
- TITAN GEL High Resolution Protein Buffer (2 pkg.)
- TITAN GEL High Resolution Protein Stain (1 vial)
- TITAN GEL Blotter A (20)
- TITAN GEL Wicks (75)
- TITAN GEL HR Templates (10)

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

Item	Cat. No.
Amido Black Protein Stain	3048
TITAN GEL Chamber Cooling Device	3039
Dialamatic Microdispenser and Tubes	6210
TITAN GEL Chamber	4063
I.O.D. (Incubator, Oven, Dryer)	5116
Titan Plus Power Supply	1504
EWS Digital Power Supply	1520
TITAN GEL Multi-Staining Set	1558
TITAN GEL High Resolution Protein Marker	5141
EWC (Electrophoresis Work Center) (Includes electrophoresis chamber, incubation chamber, dryer and power supply)	1551
TITAN GEL Chamber Adaptor for EWC	1559

Materials needed but not provided:

0.85% Saline
Methanol
Glacial Acetic Acid

Fixative-Destaining Solution: Mix 500 mL methanol with 500 mL deionized water. Acidify with 100 mL of glacial acetic acid. Store at 15 to 30°C.

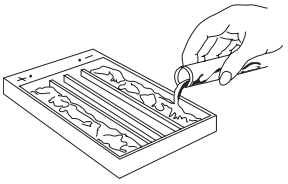
SUMMARY OF CONDITIONS

Gels	TITAN GEL High Resolution Protein Gel
Buffer Dilution	Dissolve in 1500 mL water
Sample Volume	2.0 µL
Sample Absorption Time	5 minutes
Electrophoresis Time	20 minutes
Voltage	250V
Staining Time	15 minutes
Drying Time (after staining)	10 minutes at 60-70°C
Destaining Time	2 washes, 15-30 seconds each
Drying Time	5 minutes at 60-70°C

STEP-BY-STEP METHOD

A. Preparation of the TITAN GEL CHAMBER

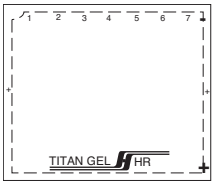
1. Dissolve one package TITAN GEL High Resolution Buffer in 1500 mL deionized water. Buffer requires approximately 20 minutes for dissolution.
2. Place the TITAN GEL Chamber Cooling Device in the refrigerator at least 1 hour prior to placing it in the TITAN GEL Chamber. Do not place it in the chamber until immediately before placing the gel in the chamber. DO NOT FREEZE the Cooling Device. NOTE: If the EWC is used as a power supply for electrophoresis, the TITAN GEL Chamber/Adaptor (Cat. No. 1559) must be used to plug into the EWC.



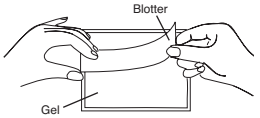
3. Pour approximately 150 mL of buffer into each of the outer sections of the chamber.
4. Cover the chamber until ready to use.

B. Sample Application

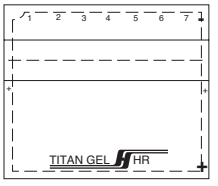
1. Prepare patient samples according to instructions in SPECIMEN COLLECTION AND HANDLING.
2. Remove the TITAN GEL High Resolution Protein Gel from the protective packaging. One edge of the agarose gel has been numbered for easy sample placement and identification.



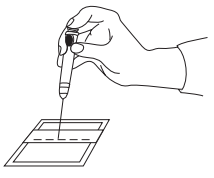
3. Using TITAN GEL Blotter A, gently blot the gel at the area of application using slight fingertip pressure on the blotter.



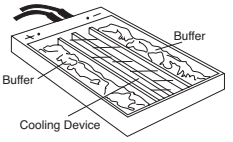
4. Carefully place the TITAN GEL HR Template on the gel aligning the application slits with the minus signs (-) on the sides of the gel and trying to avoid trapping any air bubbles under the template. Place a Blotter A over the template and remove any bubbles in the slit area with slight fingertip pressure. Retain the blotter for use in Step 8.



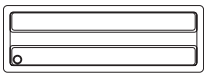
5. Place 2.0 µL of each sample (prepared according to instructions) on the template slits, spreading the sample over the entire slit. Apply the samples as quickly as possible. A 4.0 µL sample may be applied, if darker bands are desired.



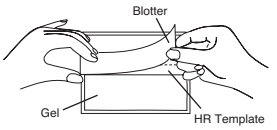
6. Wait 5 minutes after the last sample has been applied to allow the samples to diffuse into the agarose.



7. Just prior to placing the gel in the electrophoresis chamber, remove the cooling device from the refrigerator and place in the center of the chamber. Wet the entire surface of the cooling device with a few drops of buffer. Cover the chamber.

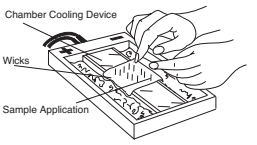


8. Gently blot the template with the Blotter A retained in Step 4 and then carefully remove the blotter.
9. Wait 30 seconds and then carefully remove the template.



C. Electrophoresis of the Sample Gel

1. Quickly place the gel into the chamber laying it, agarose side up, on top of the chamber cooling device. Position the gel so that the application point is on the cathodic (-) side. Take care to avoid trapping air bubbles between the agarose gel and the surface of the cooling device. Run only one gel per chamber.
2. Prepare a wick for each side of the gel by placing three (3) TITAN GEL Wicks together in two sets, making two thick wicks. Evenly align the edges of each set of wicks while they are dry. Dip the wicks into the chamber buffer and remove excess buffer by squeezing them between two fingers. Then attach the wicks to each side of the gel parallel to the edge of the chamber cooling device. One edge of the wicks must be immersed in the buffer while the other edge makes contact with the edge of the agarose. Gently rub one finger across the gel at the wick contact area to insure good contact.



3. Place the cover on the chamber and allow the gel to remain in the chamber for 30-60 seconds before turning on the power. This will allow the gel to equilibrate in the buffer.
4. Electrophorese the gel at 250 volts for 20 minutes.

D. Visualization of the Protein Bands

A Fixative-Destaining Solution is required for each of the staining methods. Mix 500 mL methanol with 500 mL deionized water. Acidify with 100 mL glacial acetic acid. The solution may be stored at 15 to 30°C.

1. Recommended Staining Procedure

TITAN GEL High Resolution Stain - *TO BE USED FOR SERUM, PLASMA, CSF OR URINE SAMPLES.*

a. At the end of the electrophoresis period, remove the gel from the chamber and place it in the stain agarose side up, for 15 minutes. Agitate or use a rotator during staining.
b. Remove the gel from the stain and place it in the Destain solution for 30 seconds. Agitate or swirl the solution during this time.
c. Remove the gel from the destain and place it in an I.O.D. or other laboratory drying device with forced air at 60-70°C for 5 minutes or until dry. The gel will properly destain only if completely dry at this point. The gel may be dried at a lower temperature but additional time will be required. When completely dry, the gel will be flat and will have an even sheen.
d. Destain the gel further by placing it, agarose side up, in two (2) consecutive washes of Fixative-Destaining solution. Agitate to aid in destaining. Allow the gel to remain in each wash for 30 seconds. A final water wash may be used if trace amounts of background stain remain in the gel. To remove the stain from the back of the gel, wipe the back with a laboratory tissue dampened in methanol.
e. Dry the destained gel at 60-70°C for five (5) minutes, or until dry.

Optional:
If the gel is still not clear, place it in the Destain solution until clear. Quickly dip the gel into a water wash and dry it again at 60-70°C for five minutes or until dry.

f. Visually inspect the gel for the presence of the protein bands.

2. Alternate Staining Procedures

Two staining protocols are presented in this section. The method of choice depends on the type of staining results desired by the individual user.

The coomassie stains have more sensitivity. The amido black stain is less sensitive, but provides better staining uniformity for qualitative comparison of band intensity due to differences in protein concentrations. A clearer background may be obtained with the Amido Black Stain. The double staining procedure using both a coomassie stain and amido black may provide clearer visualization of the urine protein bands and cerebrospinal fluid.

a. Amido Black Stain - *TO BE USED FOR UNDILUTED SERUM/PLASMA*
   (1) At the end of the electrophoresis period, remove the gel from the chamber and place it in the stain, agarose side up, for 10 minutes.
   (2) Wash off the excess stain by dipping the gel into the Fixative-Destaining Solution for 30 seconds.
   (3) Place the gel in an I.O.D. or other laboratory drying device with forced air at 60-70°C for 10 minutes or until dry. The gel will properly destain only after being completely dry. The gel may be dried at a lower temperature but additional time will be required. When completely dry, the gel will be flat and will have an even sheen.
   (4) Destain the gel by placing it, agarose side up, in two (2) consecutive washes of Fixative-Destaining Solution. Agitate to aid in destaining. Allow the gel to remain in each wash for 30 seconds.
   A final water wash may be used if trace amounts of background stain remain in the gel. To remove the stain from the back of the gel, wipe the back with a laboratory tissue dampened in methanol.
   (5) Dry the gel in an oven or I.O.D. at 60-70°C for five (5) minutes or until dry.
   (6) Visually inspect the gel for the presence of the protein bands.

b. Double Staining Technique using the combination of Amido Black Stain and TITAN GEL High Resolution Stain. *TO BE USED FOR URINE AND CEREBROSPINAL FLUID SAMPLES ONLY.*
   (1) Stain the gel with Amido Black Stain as outlined in the above procedure.
   (2) After completion of Step a.(5). of the amido black procedure, stain the gel with TITAN GEL High Resolution Stain using the exact procedure outlined for amido black, but substituting the coomassie stain for amido black.

Stability of End Product:

The completed dried TITAN GEL High Resolution Protein Gel is stable for an indefinite period of time.

Quality Control:

The TITAN GEL High Resolution Protein Marker (Cat. No. 5141) may be used to verify appropriate protein band separation and stain sensitivity. Refer to the package insert for more information.

RESULTS

Plasma Proteins

Figure 1 illustrates the migration patterns of 15 plasma proteins which may be identified using the TITAN GEL High Resolution Protein Procedure.

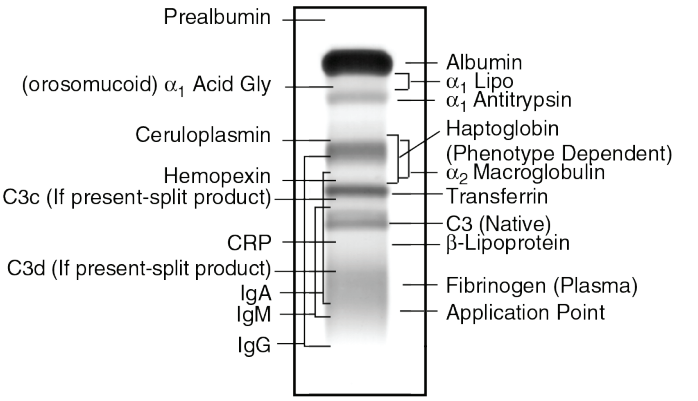


Figure 1: Illustration of relative band positions.

Urine Proteins

An electrophoretic pattern of normal urine will show a trace of albumin and sometimes a faint transferrin band.

The urine pattern in glomerular proteinuria usually consists of strong bands of albumin, both α_1-acid glycoprotein and α_1-antitrypsin in a broad α_1 zone, and transferrin β_1 region. The serum pattern shows marked decreases in these proteins with increases in the large proteins which are retained by the glomerulus.

The urine pattern in tubular proteinuria usually consists of a faint albumin band, a double band in the α_2 region due to α_2-microglobulin, a strong band in the mid-beta region due to β_2-microglobulin and some-times diffuse background staining in the gamma region due to free light chains.

Chronic renal disease or renal failure can lead to damage of both glomerulus and tubules. This results in a combined pattern with both "glomerular type" and "tubular type" proteins appearing in the urine.

Cerebrospinal Fluid Proteins

When CSF is concentrated 80 to 100 fold, the pattern from a normal adult shows a prominent pre-albumin fraction that migrates slightly faster than plasma prealbumin. Albumin is the major band on electrophoresis, comprising from 55 to 75% of the normal CSF protein. The α_1 band consists primarily of α_1-antitrypsin, the α-lipoprotein fraction being greatly decreased. The α_2 region is not a dominant fraction, as with plasma, owing to relative decreases in large proteins such as α_2-macroglobulin and the polymeric haptoglobin phenotypes. Transferrin is detected in the β_1 region and the major β_2 protein is a carbohydrate-deficient "CSF-specific" transferrin. The gamma region, consisting almost exclusively of immunoglobulin G can show some very faint banding in normal samples. The cathodal end of this zone often contains a low-M_r non-immunoglobulin protein, referred to as gamma trace, which is perhaps synthesized within the central nervous system, but has undetermined clinical significance.

LIMITATIONS

High resolution protein electrophoresis is less sensitive than isoelectric electric focusing with IgG immunoblotting for detection of oligoclonal bands.[9,10,11,12] Helena Laboratories SPIFE IgG Isoelectric Focusing (Cat. No. 3385 or 3389) is recommended for screening and diagnosis of multiple sclerosis.

Aging of serum samples will cause the C_3 band to migrate in the transferrin region. Fresh specimens only should be tested. Samples should be at room temperature before use to prevent cryoprecipitation at the application point.

INTERPRETATION OF RESULTS

Serum/Plasma

High resolution protein electrophoresis patterns are primarily interpreted by comparing the relative intensities of the bands obtained on unknown specimens with those obtained on known normal individuals. One of the most common abnormal serum protein patterns is that observed in the non-specific inflammatory response which is characterized by an increase

in α_1-antitrypsin and haptoglobin with decreased prealbumin, albumin and transferrin. While it is not useful in establishing a general diagnosis, it is useful in monitoring a patient's response to therapy. Other examples of clinically important variations are:

- elevation of the transferrin band suggesting a low level of iron
- presence of monoclonal proteins suggesting abnormalities of the immune system
- low haptoglobin suggesting elevated RBC turnover or in-vitro hemolysis
- CRP presence indicating an acute inflammatory response
- low prealbumin, albumin and transferrin with diffuse hypergam-maglobulinemia suggesting chronic inflammation, infection or antigenic stimulation
- low C_3 on fresh samples suggesting complement consumption.

CSF

Oligoclonal immunoglobulin patterns can be seen in both serum and cerebrospinal fluid as faint bands in the gamma zone. They are usually multiple homogeneous, narrow and discrete in appearance. When seen in serum, the bands indicate the presence of immune complexes which are associated with Hodgkins disease or a non-specific early immune response to various disease states. In the cerebrospinal fluid their presence is supportive evidence for the diagnosis of multiple sclerosis (MS) in the proper clinical setting. Oligoclonal bands in serum are not indicative of MS. An oligoclonal pattern in the CSF is present in more than 90% of MS patients at some time during the course of the disease. It may be less sensitive during an initial attack and is not completely specific for MS. **High resolution protein electrophoresis is less sensitive than isoelectric electric focusing with IgG immunoblotting for detection of oligoclonal bands.**[9,10,11,12] Other diseases commonly associated with an oligoclonal pattern include subacute sclerosing panencephalitis and some infections of the central nervous system.[13,14]

Urine

High resolution protein electrophoresis is an excellent analytical technique to gain a broad overview of urine proteins.[3,8] Glomerular-type proteinuria, tubular-type proteinuria as well as mixed glomerular-tubular patterns and the various overflow states can be easily distinguished and characterized, thus providing useful information on specific functions within the nephron.

The Helena publications, "HIGH RESOLUTION PROTEIN ELECTROPHORESIS, A Clinical Overview with Case Studies" and "Introduction to High Resolution Protein Electrophoresis and Associated Techniques" provide a complete discussion of the uses and interpretation of high resolution protein electrophoresis. To obtain your copy call Helena Laboratories' Literature Distribution Center using the toll free number listed at right.

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