

SPIFE™ SPE Hi Res-15 Procedure

Cat. No. 3430

Helena Laboratories

The Helena SPIFE High Resolution Protein System is intended for the qualitative separation of protein fractions in serum or urine using 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. SPIFE SPE Hi Res-15 Template Gel

Ingredients: Each gel contains agarose in Tris barbital buffer with sodium azide added as a preservative.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST. The gel 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: 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, (4) thinning of the gel blocks.

2. Acid Violet Stain

Ingredients: The stain contains Acid Violet.

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

Preparation for Use: Dissolve the dry stain in 1 L 10% acetic acid. Filter before use if necessary.

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.

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

INSTRUMENT

A SPIFE Analyzer (Cat. No. 1109) must be used to electrophorese, stain and destain the gel. Refer to the appropriate Operator's Manual for detailed instructions

SPECIMEN COLLECTION AND HANDLING

Specimen: The specimen may be serum or urine. Specimen should be free of hemolysis or lipemia. A fibrinogen band, which may obscure the beta-gamma zone, will appear in plasma samples.

Specimen Preparation:

Serum: Dilute serum samples 1:8 with 0.85% saline solution (1 part serum and 7 parts 0.85% saline solution).

Urine: Shake samples to homogenize. Centrifuge desired volume at 2000 x g for 5 minutes. Remove supernatant and concentrate as follows:

Total Protein (mg/dL)	Conc. Factor
< 50	100x
50-100	50x
100-300	25x
300-600	10x
> 600	5x

Storage: Fresh serum is the specimen of choice. If storage is necessary, samples may be stored covered at 2 to 6°C for 48 hours.

PROCEDURE

Materials provided: The following materials needed for the procedure are contained in the SPIFE SPE Hi Res-15 Template Kit (Cat. No. 3430). Individual items are not available.

- SPIFE SPE Hi Res-15 Template Gels (10)
- Acid Violet Stain (1 vial)
- REP Blotter C (10)
- Blotter A-Plus (10)
- REP SPE Hi Res-15 Templates (10)

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

Item	Cat. No.
High Resolution Protein Marker	5141
REP Prep	3100
REP Alignment Tray	

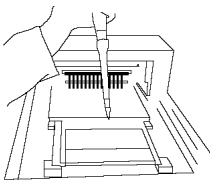
Materials needed but not provided:

- Item**
- Glacial Acetic Acid - 10%
- Destaining Solution: Glacial Acetic Acid - 5%
- 0.85% Saline Solution

STEP-BY-STEP METHOD

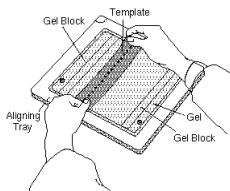
A. Sample Application

- Dilute each serum sample 1:8 with 0.85% saline solution (1 part sample + 7 parts 0.85% saline solution). The High Resolution Marker should be diluted 1:4 with saline. Urine samples should be concentrated as described in Specimen Preparation.



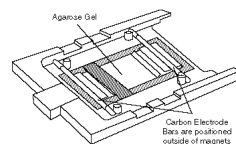
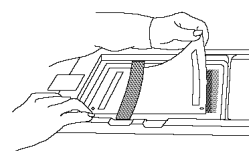
B. Gel Preparation

- Press the **TEST SELECT** button located on the electrophoresis chamber side of the instrument until the **HIGH RESOLUTION** option appears on the display.
- Dispense approximately 2 mL of REP Prep onto left side of SPIFE chamber.
- Remove the gel from the protective packaging and discard overlay.
- Position the Alignment Tray vertically so that the notched corner is at the upper right. Carefully place the left edge of the gel over the large aligning pins on the left side of the Alignment Tray.
- Using a REP Blotter C, gently blot the entire gel using slight fingertip pressure on the blotter. Remove the blotter, and place the sample template on the gel, aligning the application slits with the upper set of pins on the sides of the Alignment Tray. The templates have been marked with a hole in one corner. Place the marked corner in the lower left position. Apply slight fingertip pressure to the template, making sure there are no air



bubbles under it.

- Carefully remove the gel from the tray, and place the left edge of the gel over REP Prep aligning the round hole on the left pin of the chamber. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right side, fitting the obround hole over the right pin. Use paper towel or absorbent paper to wipe around the edges of the plastic gel backing, especially next to electrode posts to remove excess REP Prep. Make sure that the gel lays flat in the chamber and that no bubbles remain under the gel.
- Clean the electrodes with deionized water and wipe with lint-free tissue before and after each use.
- Place a carbon electrode on the outside ledge of each gel block outside the magnetic posts.
- Using the instructions provided in the Operator's Manual, set up parameters as follows:



Electrophoresis Unit

1) Apply 1	3:00	21°C	
Apply sample to template, (continue)			
2) Electrophoresis 1	18:00	17°C	525 V
Blot and remove template, (continue)			
3) Dry 1	12:00	54°C	
Remove gel blocks, (continue)			
4) END of test			
No Prompt			

Stainer Unit

1) Stain 1	4:00	Recirculate ON
No Prompt		
2) Destain 1	3:00	Recirculate ON
No Prompt		
3) Destain 2	2:00	Recirculate ON
No Prompt		
4) Destain 3	1:00	Recirculate ON
No Prompt		
5) Dry 1	12:00	63°C
No Prompt		
6) END of test		
No Prompt		

B. Sample Application

- Apply 3 µL of sample or Marker to each slit in the template.
- Close the chamber lid and press the **START/STOP** button. Sample application will be timed for 3 minutes.
- After sample application is complete, blot the template with a Blotter A-Plus to remove any excess sample. Remove the template and blotter, and discard as a biohazard.
- Close the chamber lid and press the **TEST SELECT** button to continue with electrophoresis.

- After electrophoresis is complete, use the Gel Block Remover to remove the gel blocks.
- Close the chamber lid and press the **TEST SELECT** button to dry the gel.
- After the gel has been dried, carefully remove the gel from the electrophoresis chamber.

D. Stain/Destain

- Remove the Gel Holder from the Stainer Chamber. Attach the gel to the holder by placing the round hole on the gel mylar over the left pin on the holder and the obround hole on the right pin on the holder. The gel must face backward in the Stainer Chamber.
- Place the Gel Holder with attached gel into the Stainer Chamber.
- Press the **TEST SELECT** button until **HIGH RESOLUTION** appears on the display.
- Press the **START/STOP** button to begin the staining process. The instrument will stain, destain, and dry the gel.
- SPIFE will beep when staining is complete, remove the gel and scan in a densitometer.

Stability of End Product:

The completed dried SPIFE SPE Hi Res-15 Template Gel is stable for an indefinite period of time.

Quality Control:

The 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 shows the separation typically seen using the SPIFE High Resolution Protein Procedure. Figure 2 shows the relative position of most of the plasma proteins.



Figure 1: A SPIFE SPE Hi Res-15 gel

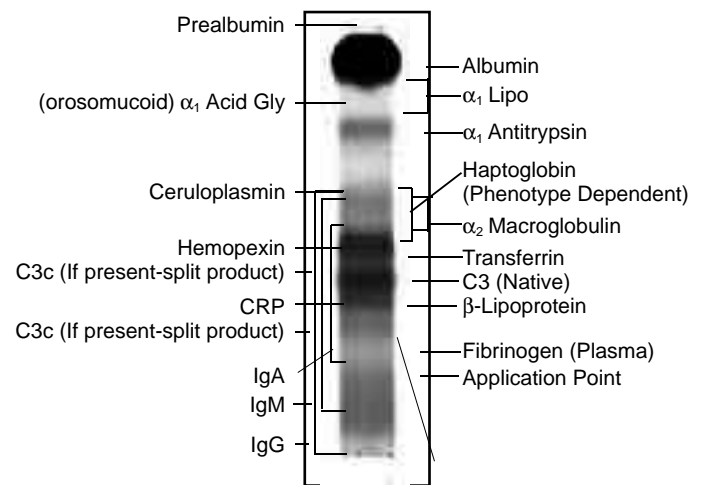


Figure 2: Illustration of relative band positions

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 sometimes 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.

LIMITATIONS

Aging of serum samples will cause the C_3 band to migrate in the transferrin region. Fresh specimens only should be tested, and they should not be hemolyzed or lipemic. Samples should be at room temperature before use to prevent cryoprecipitation at the application point. Gels which do not lay flat in the chamber, or those with surface artifacts, should not be used.

INTERPRETATION OF RESULTS

Serum

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 hypergammaglobulinemia, suggesting chronic inflammation, infection or antigenic stimulation
- low C₃ on fresh samples, suggesting complement consumption.

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.

BIBLIOGRAPHY

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SPIFE SPE Hi Res Template System
SPIFE SPE Hi Res-15 Template Kit Cat. No. 3430

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- REP Blotter C (10)
- Blotter A-Plus (10)
- SPE Hi Res-15 Templates (10)

Other Supplies and Equipment

The following items, needed for performance of the SPIFE SPE Hi Res-15 Template Procedure, must be ordered individually.

	Cat. No.
SPIFE Analyzer	1109
High Resolution Marker	5141
REP Prep Solution	3100

For Sales, Technical and Order Information, and Service Assistance, call 800-231-5663 toll free.

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