SPIFE® 3000 SPE Hi Res-20 Procedure for Plastic Applicators

Cat. No. 3430

The SPIFE 3000 SPE Hi Res-20 method is intended for the qualitative separation of protein fractions in serum, cerebrospinal fluid (CSF) or urine using agarose gel electrophoresis on the SPIFE 3000 system.

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 to 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.

COMPONENTS

1. **SPIFE SPE Hi Res Gel**

   Ingredients: Each gel contains agarose in Tris barbital buffer, sorbitol and a preservative.

   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST. The gel 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, (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, then fill the stain vat.

   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 stain solution is stable for six months when stored at 15 to 30°C in a closed container.

   Signs of Deterioration: The diluted stain should be an homogeneous mixture free of precipitate. This stain must be replaced after processing 10 gels to avoid contamination. This stain must be replaced after processing 10 gels to avoid contamination.

3. **Citric Acid Destain**

   Ingredients: After dissolution, the destain contains 0.3% (w/v) citric acid.

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

   Preparation for Use: Pour 11 L of deionized water into the Destain vat. Add the entire package of Destain. Mix well until completely dissolved.

   Storage and Stability: Store the Destain at 15 to 30°C. It is stable until the expiration date on the package.

   Signs of Deterioration: Discard if solution becomes cloudy.

INSTRUMENT

A SPIFE 3000 Analyzer must be used to apply sample, electrophorese, stain, destain and dry the gel. Refer to the appropriate Operator's Manual for detailed instructions.

SPECIMEN COLLECTION AND HANDLING

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

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

Specimen Preparation:

Serum: Dilute serum samples 1:2 with 0.85% saline solution (1 part serum and 1 part 0.85% saline solution).

Urine: Urine samples may be run neat, diluted or concentrated. One application or three applications can be made to obtain desired intensity. To concentrate 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

CSF: CSF samples may be used after proper concentration of 10-50x.

PROCEDURE

Materials provided: The following materials needed for the procedure are contained in the SPIFE SPE Hi Res-20 Kit (Cat. No. 3430). Individual items are not available.

- SPIFE SPE Hi Res-20 Gels (10)
- Acid Violet Stain (1 vial)
- REP Blotter C (10)
- Citric Acid Destain (1 pkg)
- Blade Applicator Kit (10)

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

Item	Cat. No.
SPIFE 3000 Analyzer	1088
Hi Res Protein Marker	5141
Gel Block Remover	1115
REP Prep	3100
Applicator Blade Weights	3387
Disposable Sample Cups	3369
SPIFE Dispo Cup Tray	3370

Materials needed but not provided:

- 10% Acetic Acid
- 0.85% Saline Solution

STEP-BY-STEP METHOD

I. Sample Preparation

No. of Applications	Serum	Urine	CSF	Hi Res Marker
1	1:2 with saline	neat,diluted or concentrated	———	neat
3	———	neat,diluted or concentrated	10-50x	1:3 with saline

1. **Serum**
   Dilute each serum sample 1:2 with 0.85% saline solution (1 part serum and 1 part 0.85% saline solution). The Hi Res Marker should be run neat. Only one application is used.
2. **Urine**
   Urine samples may be run neat, diluted or concentrated as described in "**Specimen Preparation**". One application of urine can be run on the same gel with serum samples. If one application of urine is used, the marker should be run neat.
   However, if three applications of urine are used, the marker should be diluted 1:3 with saline. Three applications of urine cannot be run with the serum specimens.
3. **CSF**
   CSF samples should be concentrated 10-50x. The marker should be diluted 1:3 with saline. Three applications of CSF are needed and cannot be run with serum specimens.

II. Instrument Setup

1. Remove one Disposable Applicator Blade from the packaging.
2. Place the Applicator Blade into the vertical slot numbered 8 in the Applicator Assembly.
   NOTE: The Applicator Blade will only fit into the slots in the Applicator Assembly one way; do not try to force the Applicator Blade into the slots.
3. Place an Applicator Blade Weight on top of the Applicator Blade. When placing the weight on the blade, position the weight with the thick side to the right.
4. Slide the Disposable Sample Cups into the middle row, numbered 21 to 40, of the Cup Tray.
5. Pipette 20 µL of the appropriate sample into the cups. Cover the tray until ready for use.

III. Gel Preparation

1. Remove the gel from the protective packaging and discard overlay.
2. Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.
3. Place the left edge of the gel over the 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, fitting the obround hole over the right pin. Use lint-free tissue to wipe around the edges of the plastic gel backing, especially next to electrode posts, to remove excess REP Prep. Make sure no bubbles remain under the gel.
4. Place a SPIFE Blotter C on the gel with the longer edge parallel with gel blocks. Gently blot the entire surface of the gel using slight fingertip pressure on the blotter, and remove the blotter.
5. Clean the electrodes with deionized water before and after each use. Wipe with a lint free tissue.
6. Place a carbon electrode on the outside ledge of each gel block outside the magnetic posts. Improper contact between electrode and the gel block may cause skewed patterns. Close the chamber lid.
7. Press the **TEST SELECT/CONTINUE** button located on the Electrophoresis and Stainer sides of the instrument until the **HIGH RESOLUTION** option appears on the display.

IV. Sample Application/Electrophoresis

Electrophoresis Unit

- **Serum and/or Urine**

1) No Prompt Load Sample 1	00:30	21°C	SPD1	
2) No Prompt Apply Sample 1	00:30	21°C	SPD1	LOC1
3) No Prompt Electrophoresis 1	18:00	17°C	525 V	90 mA
4) Remove gel blocks, (continue) Dry 1	12:00	54°C		
5) No Prompt END OF TEST				

- **Urine/CSF (3 applications)**

1) No Prompt Load Sample 1	00:30	21°C	SPD1	
2) No Prompt Apply Sample 1	00:30	21°C	SPD1	LOC1
3) No Prompt Load Sample 2	00:30	21°C	SPD1	
4) No Prompt Apply Sample 2	00:30	21°C	SPD1	LOC1
5) No Prompt Load Sample 3	00:30	21°C	SPD1	
6) No Prompt Apply Sample 3	1:00	21°C	SPD1	LOC1
7) No Prompt Absorb 1	1:00	21°C		
8) No Prompt Electrophoresis 1	18:00	17°C	525 V	90 mA
9) Remove gel blocks, (continue) Dry 1	12:00	54°C		
10) No Prompt END OF TEST				

Stainer Unit

- **Serum, CSF and Urine**

1) No Prompt Stain 1	4:00	REC = OFF	VALVE = 5
2) No Prompt Destain 1	3:00	REC = ON	VALVE = 2
3) No Prompt Destain 2	2:00	REC = ON	VALVE = 2
4) No Prompt Destain 3	1:00	REC = ON	VALVE = 2
5) No Prompt Dry 1	12:00	63°C	
6) No Prompt END OF TEST			

1. Open the chamber lid and place the Cup Tray with samples on the SPIFE 3000. Align the holes in the tray with the pins on the instrument. Close the chamber lid.
2. With the appropriate test name on the display, press the **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** button to begin. The SPIFE 3000 will apply the samples, electrophorese and beep when completed.
3. After electrophoresis is complete, open the chamber lid and use the Gel Block Remover to remove the gel blocks. Place one electrode across each end of the gel to prevent curling during drying. Dispose of blades and cups as biohazardous waste.
4. Close the chamber lid, and press the **TEST SELECT/CONTINUE** button to dry the gel.

V. Visualization

1. After the gel has been dried, open the chamber lid and carefully remove the gel from the electrophoresis chamber.
2. Remove the Gel Holder from the stainer unit. Attach the gel to the holder by placing the round hole in the gel backing over the left pin on the holder and the obround hole over the right pin on the holder.
3. Place the Gel Holder with the attached gel facing backwards into the stainer unit.
4. With the appropriate test name on the display, press the **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** to begin. The instrument will stain, destain and dry the gel.
5. When the gel has completed the process, the instrument will beep. Remove the Gel Holder from the stainer.

Stability of End Product:

The completed SPIFE 3000 SPE Hi Res-20 Gel is stable for an indefinite period of time.

Quality Control:

The Hi Res 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 relative position of most of the plasma proteins.

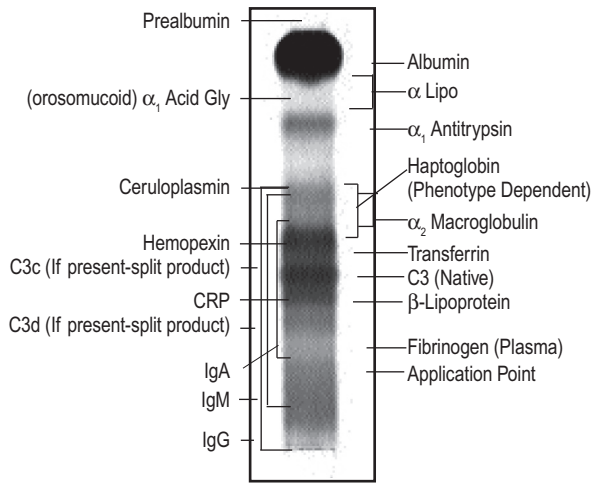

Figure 1: Illustration of relative band positions

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 in the 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.

Cerebrospinal Fluid Proteins

When CSF is concentrated, 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 Isoelectic Focusing (Cat. No. 3385 or 3389) is recommended for screening and diagnosis of multiple sclerosis. Aging of serum samples will cause the C3 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 C_3 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.

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 focusing with IgG immunoblotting for detection of oligoclonal bands.** Other diseases associated with an oligoclonal pattern, include subacute sclerosing panencephalitis and some infections of the central nervous system.[13,14]

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