SPIFE® 3000 SPE
Procedure for Plastic Applicators

Cat. No. 3460, 3461, 3462

The SPIFE 3000 SPE method is intended for the separation of serum, cerebrospinal fluid (CSF) or urine proteins by agarose gel electrophoresis using the SPIFE 3000 system.

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, with the exception of albumin, normally contains two or more components. The relative proportions of these fractions have proven to be useful aids in the diagnosis and prognosis of certain disease states.[3-5]

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 can be either polar or nonpolar at a given pH. In the SPIFE SPE procedures, proteins are separated according to their respective electrical charges on agarose gel using both the electrophoretic and electroendosmotic forces present in the system. The proteins are then stained with a visible stain.

COMPONENTS

1. **SPIFE SPE Gel**
   Ingredients: Each gel contains agarose in a tris/sodium barbital/MOPS buffer with salicylic acid, citric acid, stabilizers 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 horizontally 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 Blue Stain**
   Ingredients: When dissolved as directed, the stain contains 0.5% (w/v) acid blue 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 5% acetic acid. 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 six months when stored at 15 to 30°C.
   Signs of Deterioration: The diluted stain should be a homogeneous mixture free of precipitate. Discard if precipitate forms. 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.
4. **Acid Violet Stain (Optional Urine Stain)**
   Ingredients: The stain is comprised of Acid Violet stain.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.
   Preparation for Use: Dissolve the dry stain in 1 L of 10% acetic acid and mix thoroughly. Fill the SPIFE 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 six months when stored at 15 to 30°C in a closed container.
   Signs of Deterioration: The diluted stain should be a homogeneous mixture free of precipitate. This stain must be replaced after processing 10 gels to avoid contamination.

INSTRUMENTS

A SPIFE 3000 Analyzer must be used to electrophorese, stain, destain and then dry the gels. The gels may be scanned on a separate densitometer such as the QuickScan Touch/2000 (Cat. No. 1660/1690). Refer to the Operator's Manual for detailed instructions.

SPECIMEN COLLECTION AND HANDLING

Specimen: Fresh serum, CSF or urine is the specimen of choice. Use of plasma will cause a fibrinogen band to appear as a distinct narrow band between the beta and gamma fractions.

Storage and Stability: If storage of serum is necessary, samples may be stored covered at 15 to 30°C for 4 days, 2 to 8°C for 2 weeks or -20°C for 6 months.[6] Urine or CSF samples may be stored covered at 2 to 8°C for up to 72 hours or at -20°C for 1 month.

Urine Sample Preparation: Urine samples may be run neat, diluted or concentrated. 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 Sample Preparation: CSF samples may be used after proper concentration (10 - 50X).

Interfering Factors:

1. Inaccurate results may be obtained on specimens left uncovered, due to evaporation.
2. Hemolysis may cause false elevation in the $alpha_2$ and beta fractions.

PROCEDURE

Materials Provided: The following materials needed for the procedure are contained in the SPIFE SPE Kits. Individual items are not available.

Sample Test Size	Cat. No.
60 Sample	3460
40 Sample	3461
20 Sample	3462

SPIFE SPE Gels (10)
Acid Blue Stain (1 vial)
SPIFE Blotter C (10)
Citric Acid Destain (1 pkg)
Blade Applicator Kit

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

Item	Cat. No.
SPIFE Analyzer 3000	1088
QuickScan 2000	1660
QuickScan Touch	1690
ESH Touch	1380
Electrophoresis Auto Sample Handler	1341
Gel Block Remover	1115
SPE Normal Control	3424
SPE Abnormal Control	3425
REP Prep	3100
Applicator Blade Weights	3387
Disposable Sample Cups	3369
SPIFE 3000 Dispo Cup Tray	3370
SPIFE Urine/CSF Protein Accessory Kit	3427
SPIFE Urine IFE Alignment Tray	3380
Acid Violet Stain	552351

Materials needed but not provided:
5% acetic acid
0.85% saline

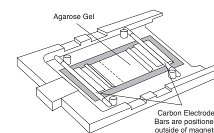
STEP-BY-STEP METHOD

I. Sample Preparation

1. If testing 41-60 samples, remove three disposable Applicator Blades from the packaging. If testing fewer than 41 samples, remove the appropriate number of Applicator Blades from the packaging
2. Place the three Applicator Blades into the vertical slots numbered 2, 8 and 14 in the Applicator Assembly. If using fewer Applicator Blades, place them into any two of the three slots noted above.
   If testing serum samples, follow the instructions marked "**• Serum**", either **Option 1** or **Option 2.** If testing serum with urine or CSF, follow instructions marked "**• Serum and Urine/CSF** **".** Serum application is made after the third urine/CSF application. Place the urine/CSF blade(s) in the Applicator Assembly. After the third urine/CSF application, place the serum blade in the correct slots and remove the urine or CSF blade(s). If testing urine/CSF only, follow the instructions marked "**• Urine/CSF** ".
   NOTE: The Applicator Blades will only fit into the slots in the Applicator Assembly one way; do not try to force the Applicator Blades into the slots.
3. Place an Applicator Weight on top of each 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 appropriate rows of the cup tray. If testing less than 41 samples, place the cups into the rows that correspond with the Applicator Blade placement.
5. Pipette 15 µL of control or serum or 20 µL of urine or CSF into Disposable Sample Cups. Cover until ready to use. Urine or CSF specimens with insufficient volumes may be run using the SPIFE Urine/CSF Protein Accessory Kit (Cat. No. 3427) and the SPIFE Urine IFE Alignment Tray (Cat. No. 3380).

II. 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 side, 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 the electrode and the gel block may cause skewed patterns. Close the chamber lid.
7. Press the **TEST SELECT/CONTINUE** buttons located on the Electrophoresis and Stainer sides of the instrument until the **SERUM/URINE PROTEIN** option appears on the display.

III. Sample Application/Electrophoresis

Using the instructions provided in the Operator's Manual, set up the parameters as follows for the SPIFE 3000:

* **Due to variations in environmental conditions, a Dry time of 10 or 12 minutes is recommended, but a range of 10 to 15 minutes is acceptable.**

** **An Apply Sample time of 3 or 30 seconds is acceptable.**

Electrophoresis Unit

• Serum Option 1

Step	Time	Temp		
1) No Prompt				
Load Sample 1	00:01	21°C	SPD1	
2) No Prompt				
Apply Sample 1	**00:30	21°C	SPD1	LOC1
3) No Prompt				
Electrophoresis 1	6:00	21°C	650V	135mA
4) Remove gel blocks, (continue)				
Dry 1	*10:00	54°C		
5) No Prompt				
END OF TEST				

• Serum Option 2

Step	Time	Temp		
1) No Prompt				
Load Sample 1	00:02	21°C	SPD1	
2) No Prompt				
Load Sample 2	00:02	21°C	SPD1	
3) No Prompt				
Load Sample 3	00:02	21°C	SPD1	
4) No Prompt				
Load Sample 4	00:30	21°C	SPD1	
5) No Prompt				
Apply Sample 1	00:30	21°C	SPD1	LOC1
6) No Prompt				
Electrophoresis 1	6:00	21°C	650V	135mA
7) Remove gel blocks, (continue)				
Dry 1	*10:00	54°C		
END OF TEST				

• Urine/CSF

Step	Time	Temp		
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	00:30	21°C	SPD1	LOC1

7) No Prompt

Electrophoresis 1	6:00	21°C	650V	135mA

8) Remove gel blocks, (continue)

Dry 1	*10:00	54°C

9) No Prompt
   END OF TEST

- **Serum and Urine/CSF**

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   00:30   21°C   SPD1   LOC1
7) To Continue, (continue)
   Load Sample 4   00:01   21°C   SPD1
8) No Prompt
   Apply Sample 4   **00:30   21°C   SPD1   LOC1
9) No Prompt
   Electrophoresis 1   6:00   21°C   650V   135mA
10) Remove gel blocks, (continue)
   Dry 1   *10:00   54°C
11) No Prompt
   END OF TEST

Stainer Unit

- **Serum, CSF and Urine**

NOTE: If testing urine samples with Acid Violet Stain, change "VALVE = 3" to "VALVE = 5" in Step 1.

1) No Prompt
   Stain 1   4:00   REC = OFF   VALVE = 3
2) No Prompt
   Destain 1   1:00   REC = ON   VALVE = 2
3) No Prompt
   Destain 2   1:00   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 the test or skip the operation will be presented. Press **START/STOP** to begin. If testing serum only or urine/CSF only, the SPIFE 3000 will apply the samples, electrophorese and beep when completed.
3. If testing serum and urine/CSF, open the chamber lid after the beep. Place an Applicator Blade into the slot for serum application and remove the urine/CSF blades. Press **TEST SELECT/ CONTINUE.**
4. 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.
5. Close the chamber lid, and press the **TEST SELECT/ CONTINUE** button to dry the gel.

IV. VISUALIZATION

1. After the gel has been dried, carefully remove the gel from the electrophoresis chamber.
2. Remove the Gel Holder from the stainer chamber. 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 chamber.
4. With 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 and scan the bands in a densitometer.

Evaluation of Fractions

Qualitative Evaluation: The urine and CSF samples run on the SPIFE SPE Gel can only be qualitatively inspected for the presence of the bands.

Quantitative Evaluation: Scan the serum samples on the SPIFE Gel in the QuickScan Touch/2000, agarose side up on the acid blue setting. A slit size of 5 is recommended.

Stability of End Product: The completed, dried SPIFE SPE Gel is stable for an indefinite period of time.

Quality Control

SPE Normal Control (Cat. No. 3424) and SPE Abnormal Control (Cat. No. 3425) may be used to verify all phases of the procedure and should be used on each gel run. If desired, a control or patient sample may be diluted 1:7 with 0.85% saline (1 part sample + 6 parts saline) and run with urines and CSFs for qualitative comparison. Refer to the package insert provided with the control for assay values.

REFERENCE VALUES

The reference ranges presented were established with the SPE System on 50 normal specimens using the SPIFE Analyzers. These values are presented as a guideline.

Protein Fraction	% of Total Protein $\bar{x} \pm 2$ S.D.
Albumin	47.0 - 61.6
Alpha$_1$	2.0 - 4.4
Alpha$_2$	8.9 - 14.9
Beta	10.8 - 16.2
Gamma	9.8 - 24.4

Each laboratory should perform its own normal range study.

Variations of Expected Values[5]

Studies show that values are the same for both males and non-pregnant 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 with slightly increased alpha$_1$ and normal 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.

RESULTS

Figure 1 illustrates the electrophoretic mobilities of the albumin, $alpha_1$, $alpha_2$, beta and gamma protein bands on SPIFE SPE-60 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 $alpha_1$, followed by $alpha_2$, beta and gamma globulins.

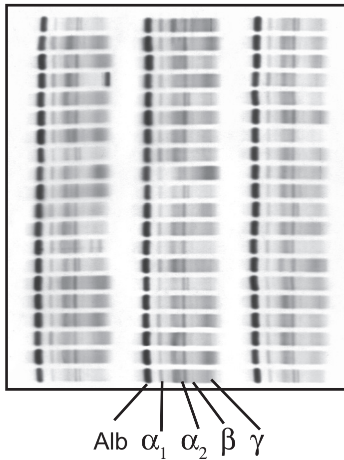


Figure 1: A SPIFE SPE-60 Gel showing relative position of the bands

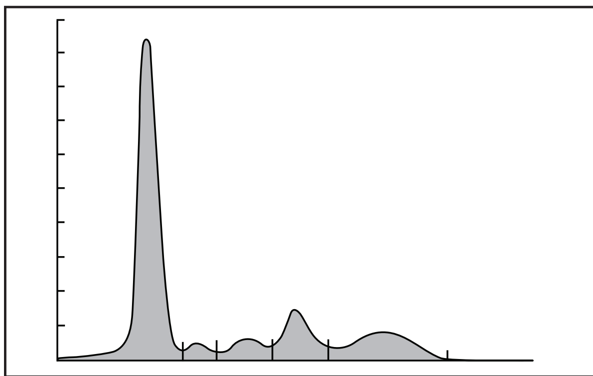


Figure 2: A scan of a SPIFE SPE pattern.

Calculations of the Unknown

The Helena QuickScan Touch/2000 densitometer will automatically calculate and print the relative percent and the absolute value of each band when the total protein is entered. Refer to the Operator's Manual provided with the instrument.

INTERPRETATION OF RESULTS[5]

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, plasma cell dyscrasias, pregnancy and genetic deficiencies.

LIMITATIONS

Since all electrophoretic procedures are nonlinear, it is critical to fill the wells with the recommended volume of appropriate sample to obtain optimal resolution and reproducible results. Noncompliance with the recommended procedure may affect the results.

SPECIFIC PERFORMANCE CHARACTERISTICS

PRECISION

Within Run: A pooled serum sample was analyzed on one gel with the following results. n = 20

Protein Fraction	Mean %	SD	CV%
Albumin	49.0	0.8	1.5
$Alpha_1$	2.9	0.2	5.3
$Alpha_2$	13.2	0.3	2.4
Beta	14.9	0.3	1.9
Gamma	20.0	0.3	1.7

Between-Run: A pooled serum sample was analyzed 20 times on each of 9 gels. n = 180

Protein Fraction	Mean %	SD	CV%
Albumin	50.6	1.1	2.2
$Alpha_1$	2.8	0.2	6.5
$Alpha_2$	13.3	0.6	4.6
Beta	13.9	0.4	2.6
Gamma	19.3	0.6	3.2

CORRELATION

Fifty normal and six abnormal serum specimens were analyzed using the SPIFE SPE with plastic blades system and the SPIFE SPE with metalized blades system.

n	= 56	
Slope	= 0.988	Y = 0.988X + 0.236
Intercept	= 0.236	X = SPIFE SPE with Metalized blades
R	= 0.9997	Y = SPIFE SPE with Plastic blades

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