SPIFE® Touch Split Beta SPE - 20, 40, 60 Procedure

Cat. No. 3422, 3421, 3420

The SPIFE Touch Split Beta SPE method is intended for the separation of serum, urine or cerebrospinal fluid (CSF) proteins by agarose gel electrophoresis using the SPIFE Touch 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 Serum Protein 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 Split Beta SPE Gel**

   Ingredients: Each gel contains agarose in a tris-barbital/MOPS buffer with calcium lactate, a stabilizer, and a preservative.

   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY.

   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, (5) crystals in gel.

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 for 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 Touch 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 a homogeneous mixture free of precipitate. This stain must be replaced after processing 10 gels to avoid contamination.

INSTRUMENTS

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

SPECIMEN COLLECTION AND HANDLING

Specimen: Fresh serum, urine or CSF 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, store serum samples 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 diluted, neat 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. 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.

PROCEDURE

Materials provided: The following materials needed for the procedure are contained in the SPIFE Split Beta SPE-20,40,60 Kits. Individual items are not available.

Test Size	Cat. No.
60 Samples	3420
40 Samples	3421
20 Samples	3422

Cat. No. 3420, 3421, 3422

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

Material provided but not contained in the kit:

ITEM	CAT. NO.
SPIFE Touch Analyzer	1068
QuickScan Touch	1690
QuickScan 2000	1660
ESH Touch	1380
Electrophoresis Auto Sample Handler	1341
Applicator Blade Weights	3387
Gel Block Remover	1115
SPE Normal Control	3424
SPE Abnormal Control	3425
REP Prep	3100
SPIFE Dispo Sample Cups	3369
SPIFE 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 Application

A. Serum, CSF and Urine (Blade Application)

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 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.
   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.
   If testing serum only, follow the instructions marked "**• Serum (Blade Application)**", either **Option 1** or **Option 2.** If testing serum with CSF or urine, follow the instructions marked "**• Serum and Urine/CSF (Blade Application)**". If testing urine/CSF only, follow the instructions marked "**• Urine/CSF (Blade Application)**".
3. Place an Applicator Blade Weight on top of each blade assembly. 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 rows of the appropriate 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.
6. Place the Cup Tray into the SPIFE Touch. Align the holes in the tray with the pins on the instrument.
7. Remove the gel from the protective packaging and discard overlay.
8. Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.
9. 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.
10. 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.
11. Clean the electrodes with deionized water before and after each use. Wipe with a lint-free tissue.
12. 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.
13. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. To check parameters, select test, press **SETUP** and proceed to Step II. Once parameters have been verified, proceed to Step III. A if running serum only or urine/CSF only or Step III. B if running serum with CSF and/or urine.

B. Urine or CSF (Template Application)

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).

1. Remove the gel from the protective packaging and discard the overlay. Carefully place the gel on the SPIFE Urine IFE Alignment Tray (Cat. No. 3380).
2. Place a SPIFE Blotter C on the gel with the longer edge parallel with the gel blocks. Gently blot the entire surface of the gel using slight fingertip pressure on the blotter, and remove the blotter.
3. The templates have been marked with a hole in one corner. One to three templates can be placed on the gel. Hold the template so that the marked corner is in the lower left position. Align the application slits with the pins on the sides of the Alignment Tray. Place the template on the gel and apply slight fingertip pressure to each template, making sure there are no air bubbles under them. Carefully remove the gel from the Alignment Tray.
4. Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.
5. 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 and ending on the right side, fitting the obround hole over the right pin. Use a 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.
6. Clean the electrodes with deionized water before and after each use. Wipe with a lint-free tissue.
7. 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 my cause skewed patterns. Close the chamber lid.
8. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. To check parameters, select test, press **SETUP** and proceed to Step II. Once parameters have been verified, proceed to Step III.C.

II. Parameters

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

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

SEPARATOR UNIT

- **Serum (Blade Application) Option 1**

Load Sample	Prompt: None Time: 0:01 Temperature: 21°C Speed: 1
Apply Sample	Prompt: None Time: 0:30* Temperature: 21°C Speed: 1 Location: 2
Electrophoresis	Prompt: None Time: 8:00 Temperature: 21°C Voltage: 650 V mA: 130 mA
Dry	Prompt: Remove Gel Blocks Time: 10:00 Temperature: 54°C
End	

- **Serum (Blade Application) Option 2**

Load Sample 1	Prompt: None Time: 0:02 Temperature: 21°C Speed: 1
Load Sample 2	Prompt: None Time: 0:02 Temperature: 21°C Speed: 1
Load Sample 3	Prompt: None Time: 0:02 Temperature: 21°C Speed: 1
Load Sample 4	Prompt: None Time: 0:30 Temperature: 21°C Speed: 1
Apply Sample	Prompt: None Time: 0:30 Temperature: 21°C Speed: 1 Location: 2
Electrophoresis	Prompt: None Time: 8:00 Temperature: 21°C Voltage: 650 V mA: 130 mA
Dry	Prompt: Remove Gel Blocks Time: 10:00 Temperature: 54°C
End	

- **Urine/CSF (Blade Application)**

Load Sample 1	Prompt: None Time: 0:30 Temperature: 21°C Speed: 1
Apply Sample 1	Prompt: None Time: 0:30 Temperature: 21°C Speed: 1 Location: 2
Load Sample 2	Prompt: None Time: 0:30 Temperature: 21°C Speed: 1
Apply Sample 2	Prompt: None Time: 0:30 Temperature: 21°C Speed: 1 Location: 2
Load Sample 3	Prompt: None Time: 0:30 Temperature: 21°C Speed: 1
Apply Sample 3	Prompt: None Time: 0:30 Temperature: 21°C Speed: 1 Location: 2
Electrophoresis	Prompt: None Time: 8:00 Temperature: 21°C Voltage: 650 V mA: 130 mA
Dry	Prompt: Remove Gel Blocks Time: 10:00* Temperature: 54°C
End	

- **Serum and Urine/CSF (Blade Application)**

Load Sample 1	Prompt: None Time: 0:30 Temperature: 21°C Speed: 1
Apply Sample 1	Prompt: None Time: 0:30 Temperature: 21°C Speed: 1 Location: 2
Load Sample 2	Prompt: None Time: 0:30 Temperature: 21°C Speed: 1
Apply Sample 2	Prompt: None Time: 0:30 Temperature: 21°C Speed: 1 Location: 2
Load Sample 3	Prompt: None Time: 0:30 Temperature: 21°C Speed: 1
Apply Sample 3	Prompt: None Time: 0:30 Temperature: 21°C Speed: 1 Location: 2
Load Sample 4	Prompt: To Continue Time: 0:01 Temperature: 21°C Speed: 1

Apply Sample 4
Prompt: None
Time: 0:30*
Temperature: 21°C
Speed: 1
Location: 2

Electrophoresis
Prompt: None
Time: 8:00
Temperature: 21°C
Voltage: 650 V
mA: 130 mA

Dry
Prompt: Remove Gel Blocks
Time: 10:00
Temperature: 54°C

End

- **Urine/CSF (Template Application)**

Pause
Prompt: None
Time: 10:00
Temperature: 21°C

Electrophoresis
Prompt: To Continue
Time: 8:00
Temperature: 21°C
Voltage: 650 V
mA: 130 mA

Dry
Prompt: Remove Gel Blocks
Time: 10:00
Temperature: 54°C

End

STAINER UNIT

- **Serum, CSF and Urine**

NOTE: If testing urines with Acid Violet Stain, change "Valve: 3" to "Valve: 5" in Stain Section.

Stain
Prompt: None
Time: 4:00
Recirculation: Off
Valve: 3
Fill, Drain

Destain 1
Prompt: None
Time: 1:00
Recirculation: On
Valve: 2
Fill, Drain

Destain 2
Prompt: None
Time: 1:00
Recirculation: On
Valve: 2
Fill, Drain

Destain 3
Prompt: None
Time: 1:00
Recirculation: On
Valve: 2
Fill, Drain

Dry
Prompt: None
Time: 12:00
Temperature: 63°C

End

III. Electrophoresis

A. Serum or Urine/CSF (Blade Application)

1. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** and choose an operation to proceed. The SPIFE Touch will apply the samples, electrophorese and beep when completed. Dispose of the blades and cups as biohazardous waste.
2. After electrophoresis is complete, open the chamber lid and use the Gel Block Remover to remove the gel blocks. Replace the electrodes on each end of the gel to prevent curling during drying.
3. Close the chamber lid and press the **CONTINUE** button to dry the gel. Proceed to Step IV.

B. Serum and Urine/CSF (Blade Application)

1. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** and choose an operation to proceed. **NOTE:** Serum and CSF or urine samples are run on the same gel on different rows. Place the Applicator Blades into the slots that correspond to the CSF or urine samples. After the third CSF/urine application, the instrument will beep and stop. Open the chamber lid, add an Applicator Blade into the remaining slot for the serum samples and remove the urine/CSF blades. Close the chamber lid and press **CONTINUE.** The instrument will apply and continue.
2. After electrophoresis is complete, open the chamber lid and use the Gel Block Remover to remove the gel blocks. Replace the electrodes on each end of the gel to prevent curling during drying.
3. Close the chamber lid and press the **CONTINUE** button to dry the gel. Proceed to Step IV.

C. Urine/CSF Template Application

1. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** to choose an operation to proceed. Open the chamber lid.
2. Apply urine and/or CSF by placing 3 µL of each sample onto one of the twenty available slits on the Urine/CSF Template.
3. Close the chamber lid, and press the **CONTINUE** button. Sample application will be timed for 10 minutes.
4. After sample application is complete, open the chamber lid and gently blot each template with a Blotter A-Plus.
5. Carefully remove the blotter(s) and template(s) and discard as biohazardous waste.
6. Close the chamber lid and press the **CONTINUE** button to begin electrophoresis. SPIFE Touch will beep when electrophoresis is complete.
7. After electrophoresis is complete, open the chamber lid and use the Gel Block Remover to remove the gel blocks. Replace the electrodes on each end of the gel to prevent curling during drying.
8. Close the chamber lid and press the **CONTINUE** button to dry the gel.

IV. 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 chamber. Attach the gel to the holder by placing the round hole over the left pin and the obround hole over the right pin.
3. Place the Gel Holder with the attached gel facing backwards into the stainer chamber.
4. Use the arrows under **STAINER UNIT** to select the appropriate test. Press **START** and choose an operation to proceed. The instrument will stain, destain and dry the gel.
5. When the process is completed, the instrument will beep. Remove the Gel Holder from the stainer and scan the bands in a densitometer.

Evaluation of the Protein Bands

1. **Qualitative Evaluation:** The urine and CSF samples run on the SPIFE Split Beta SPE Gel can only be visually inspected for the presence of the bands.

2. **Quantitative Evaluation:** Scan the SPIFE Split Beta SPE 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 Split Beta SPE Gel is stable for an indefinite period of time.

Quality Control

SPE Normal (Cat. No. 3424) and SPE Abnormal (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 Split Beta SPE System on 48 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	47.6 - 61.9
$Alpha_1$	1.4 - 4.6
$Alpha_2$	7.3 - 13.9
Beta	10.9 - 19.1
Gamma	9.5 - 24.8

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 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, with 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 16 years of age. The albumin decreases and beta globulin increases after the age of 40.

RESULTS

Figure 1 illustrates the electrophoretic mobilities of the albumin, $alpha_1$, $alpha_2$, beta and gamma protein bands on SPIFE Split Beta 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 Split Beta SPE-60 Gel showing relative position of the bands.

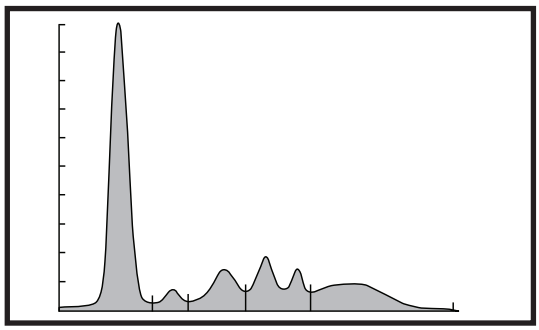

Figure 2: A scan of a SPIFE Split Beta SPE pattern.

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. Abnormal patterns are observed in pregnancy and in disorders including inflammatory response, rheumatic disease, liver diseases, protein-loss disorders, plasma cell dyscrasias and genetic deficiencies.

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.

LIMITATIONS

Since all electrophoretic procedures are nonlinear, it is critical to fill the wells with 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: A normal and an abnormal control were run alternately 20 times each on a single gel with the following results:

Normal Control (n = 20)

Protein Fraction	Mean %	SD	CV
Albumin	55.5	0.9	1.6%
$Alpha_1$	3.9	0.2	6.2%
$Alpha_2$	9.1	0.3	3.5%
Beta	16.0	0.3	2.1%
Gamma	15.4	0.5	3.4%

Abnormal Control (n = 20)

Protein Fraction	Mean %	SD	CV
Albumin	48.4	0.6	1.2%
$Alpha_1$	3.5	0.2	5.7%
$Alpha_2$	8.5	0.2	2.3%
Beta	12.5	0.2	1.7%
Gamma	27.1	0.3	1.3%

Between-Run: A normal and an abnormal control were run alternately 20 times each on nine gels with the following results:

Normal Control (n = 180)

Protein Fraction	Mean %	SD	CV
Albumin	55.5	1.1	2.0%
$Alpha_1$	3.8	0.3	7.8%
$Alpha_2$	9.3	0.3	3.5%
Beta	16.1	0.3	2.1%
Gamma	15.3	0.6	3.7%

Abnormal Control (n = 180)

Protein Fraction	Mean %	SD	CV
Albumin	48.5	0.9	1.9%
$Alpha_1$	3.4	0.2	6.7%
$Alpha_2$	8.6	0.3	3.1%
Beta	12.4	0.3	2.5%
Gamma	27.0	0.6	2.1%

CORRELATION

Normal and abnormal specimens were analyzed using the SPIFE Split Beta SPE system and the SPIFE Touch Split Beta SPE system.

n = 30

Y = 0.9968X + 0.0667

R = 0.9999

X = SPIFE Split Beta SPE

Y = SPIFE Touch Split Beta SPE

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