

# SPIFE® Split Beta SPE Procedure

Cat. No. 3422, 3421, 3420, 3422U, 3421U, 3420U

Helena  Laboratories

The SPIFE Split Beta SPE System is intended for the separation of serum, urine or cerebrospinal fluid (CSF) 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.<sup>1</sup> Since the introduction of moving-boundary electrophoresis by Tiselius<sup>2</sup> 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<sub>1</sub>, alpha<sub>2</sub>, 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.<sup>3-5</sup>

## 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 electroosmotic forces present in the system. The proteins are then stained with a visible stain.

## REAGENT

### 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.

### 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.

### 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 15-30°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 liter 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.

## INSTRUMENT

A SPIFE 3000 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 2000 (Cat. No. 1660). Refer to the Operator's Manual 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, samples may be stored covered at 15 to 30°C for 4 days or 2 to 8°C for 2 weeks, or -20°C for 6 months.<sup>6</sup> 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<sub>2</sub> and beta fractions.
2. Inaccurate results may be obtained on specimens left uncovered, due to evaporation.

## PROCEDURE

**Materials provided:** The kits can be ordered according to the matrix being tested. The applicator blade in the kit used for serum is different from the blade used for urine and/or CSF.

	Cat. No.	
Test Size	Serum	Urine/CSF
60 Samples	3420	3420U
40 Samples	3421	3421U
20 Samples	3422	3422U

The following materials needed for the procedure are contained in the SPIFE Split Beta SPE 20/40/60 Kits. Individual items are not available.

**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)  
 Modified Applicator Blade  
 Assembly-20 Sample

**Cat. No. 3420U, 3421U, 3422U**

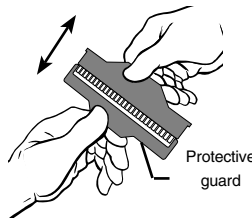
SPIFE Split Beta SPE Gels (10)  
 Acid Blue Stain (1 vial)  
 SPIFE Blotter C (10)  
 Citric Acid Destain (1 pkg)  
 SPIFE Applicator Blade  
 Assembly-20 Sample

**Material provided but not contained in the kit:**

ITEM	CAT. NO.
SPIFE 3000 Analyzer	1088
QuickScan 2000	1660
Electrophoresis Sample Handler	1341
Applicator Blade Weights	3387
Applicator Blades (for Urine & CSF)	3450
Gel Block Remover	1115
SPE Normal	3424
SPE Abnormal	3425
REP Prep	3100
SPIFE Dispo Sample Cups (deep well)	3360
SPIFE Dispo Sample Cups (for Urine & CSF)	3369
SPIFE 2000/3000 Dispo Cup Tray	3370
SPIFE Urine/CSF Protein Accessory Kit	3427
SPIFE Urine IFE Alignment Tray	3380
Acid Violet Stain	551758

**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. Remove the protective guard from the blades by gently bending the protective piece back and forth until it breaks free.



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.

**Please note that the blade assembly 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 only serum samples, follow the instructions marked "•Serum". If testing serum with urine or CSF, follow instructions marked "•Serum and CSF or Urine". Serum application is made after the second urine or CSF application. Therefore the blade for serum application is not added until after the second urine/CSF application.

3. Place an Applicator Blade Weight on top of each blade assembly.
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 the following amount of sample into the cups. Cover the tray until ready to use.

**NOTE: Application of Urine and CSF samples cannot be done with the Applicator Blades or Cups packaged in the kit. Other Blades (Cat. No. 3450) and Cups (Cat. No. 3369) must be purchased.**

Sample	Volume	Blades	Cups
Serum or control	45 µL	3451	3360
Urine or concentrated CSF	20 µL	3450	3369

Specimens with insufficient volumes may be run using the SPIFE Urine/CSF Accessory Kit (Cat. No. 3427) and the SPIFE Urine IFE Alignment Tray (3380).

**II. Gel Preparation**

1. Remove the gel from the protective packaging and discard overlay.
2. Using a SPIFE Blotter C, gently blot the entire gel using slight fingertip pressure on the blotter. Remove the blotter.
3. Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.
4. 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.
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.
7. Press the **TEST SELECT/CONTINUE** button located on the electrophoresis side of the instrument until the **SERUM/URINE PROTEIN** option appears on the display.

**III. Electrophoresis**

**NOTE: A "Dry 1" time of 10 or 12 minutes is recommended. However, due to variations in environmental conditions, the following ranges are acceptable.**

**\*Dry 1 = 10-15 minutes.**

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

**Electrophoresis Unit****• Serum**

- 1) No Prompt  
Load Sample 1 00:30 21°C SPD1
- 2) No Prompt  
Apply Sample 1 01:00 21°C SPD1 LOC2
- 3) No Prompt  
Electrophoresis 1 8:00 21°C 650 V 130 mA
- 4) Remove gel blocks, (continue)  
Dry 1 \*10:00 54°C
- 5) No prompt  
END OF TEST

**• Serum and CSF or Urine**

- 1) No Prompt  
Load Sample 1 00:30 21°C SPD1
- 2) No Prompt  
Apply Sample 1 00:30 21°C SPD1 LOC2
- 3) No Prompt  
Load Sample 2 00:30 21°C SPD1
- 4) No Prompt  
Apply Sample 2 00:30 21°C SPD1 LOC2
- 5) To Continue, (continue)  
Load Sample 3 00:30 21°C SPD1
- 6) No Prompt  
Apply Sample 3 01:00 21°C SPD1 LOC2
- 7) No Prompt  
Absorb 1 01:00 21°C
- 8) No Prompt  
Electrophoresis 1 - 8:00 21°C 650 V 130 mA
- 9) Remove gel blocks, (continue)  
Dry 1 \*10:00 54°C
- 10) No prompt  
END OF TEST

**Stainer Unit****• Serum and CSF or 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. Place the Cup Tray with samples on the SPIFE 3000. Align the holes in the tray with the pins on the instrument.
2. With **SERUM/URINE PROTEIN** 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. If testing serum only, the SPIFE 3000 will apply the samples, electrophorese and beep when completed. Dispose of blades and cups as biohazardous waste.
3. If testing serum and urine/CSF, open the chamber lid after the beep. Place the Modified Blade in the Applicator Assembly for serum application. Press **TEST SELECT/CONTINUE**.

#### IV. Visualization

1. After electrophoresis is complete, use the Gel Block Remover to remove the gel blocks. Replace the electrodes on each end of the gel to prevent curling during drying.
2. Close the chamber lid and press the **TEST SELECT/CONTINUE** button to dry the gel.
3. After the gel has been dried, carefully remove the gel from the electrophoresis chamber.
4. Remove the Gel Holder from the stainer chamber. Attach the gel to the holder by placing the round hole in the gel mylar over the left pin on the holder and the obround hole over the right pin on the holder.
5. Place the Gel Holder with the attached gel facing backwards into the stainer chamber.
6. With the **SERUM/URINE PROTEIN** prompt 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.
7. 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 at 595 nm, agarose side down on an EDC densitometer. Scan the gel agarose side up on other instruments. A slit size of 5 is recommended. If a QuickScan 2000 is used, scan on the acid blue setting.

**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 range presented was established with the Split Beta SPE System on 48 normal specimens using the SPIFE 3000 Analyzer. These values are presented as a guideline.

Protein Fraction	% of Total Protein
	$\bar{X} \pm 2 \text{ S.D.}$
Albumin	47.6 - 61.9
Alpha <sub>1</sub>	1.4 - 4.6
Alpha <sub>2</sub>	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<sup>5</sup>

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<sub>2</sub>, and beta fractions with slightly increased alpha<sub>1</sub> 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<sub>1</sub>, alpha<sub>2</sub>, 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<sub>1</sub>, followed by alpha<sub>2</sub> globulin, beta and gamma globulins.

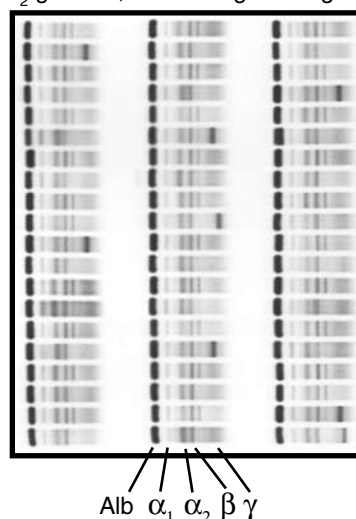


Figure 1: A SPIFE Split Beta SPE-60 Gel showing relative position of the bands.

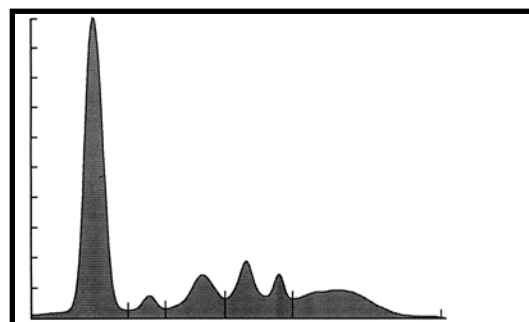


Figure 2: A scan of a SPIFE Split Beta SPE pattern.

## Calculations of the Unknown

The Helena QuickScan 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<sup>5</sup>

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 30 times each on a single gel with the following results: N = 30

Normal Control

Protein Fraction	Mean %	SD	CV
Albumin	58.1	1.3	2.2%
Alpha <sub>1</sub>	3.5	0.4	11.0%
Alpha <sub>2</sub>	11.2	1.0	8.9%
Beta	14.2	0.8	5.8%
Gamma	13.0	0.6	4.5%

Abnormal Control

Protein Fraction	Mean %	SD	CV
Albumin	51.6	0.8	1.6%
Alpha <sub>1</sub>	3.1	0.2	6.1%
Alpha <sub>2</sub>	8.8	0.3	3.4%
Beta	12.8	0.5	3.7%
Gamma	23.5	0.5	2.0%

**Between-Run:** A normal and an abnormal control were run alternately 30 times each on three gels with the following results: N = 90

Normal Control

Protein Fraction	Mean %	SD	CV
Albumin	57.7	1.1	2.0%
Alpha <sub>1</sub>	3.6	0.4	10.2%
Alpha <sub>2</sub>	10.7	1.2	10.9%
Beta	14.8	0.9	6.4%
Gamma	13.1	0.6	4.3%

Abnormal Control

Protein Fraction	Mean %	SD	CV
Albumin	51.5	1.0	2.0%
Alpha <sub>1</sub>	3.3	0.2	7.2%
Alpha <sub>2</sub>	8.6	0.6	6.8%
Beta	13.0	0.5	4.2%
Gamma	23.5	0.5	2.3%

## CORRELATION

Normal (N = 48) and abnormal (N = 48) serum samples were analyzed using the SPIFE SPE Vis-60 system and the SPIFE Split Beta SPE system.

N = 96

Y = 1.02X - 0.35

R = 0.99

X = SPIFE SPE Vis-60

Y = SPIFE Split Beta SPE

## BIBLIOGRAPHY

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- Tietz, N.W., ed., *Textbook of Clinical Chemistry*, 3rd ed., W.B. Saunders Co., Philadelphia, pg. 524, 1995.

## SPIFE SPLIT BETA SPE System

### 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)
- Modified Applicator Blade Assembly-20 sample

### Cat. No. 3420U, 3421U, 3422U

- SPIFE Split Beta SPE Gels (10)
- Acid Blue Stain (1 vial)
- SPIFE Blotter C (10)
- Citric Acid Destain (1 pkg)
- SPIFE Applicator Blade Assembly-20 Sample

### Other Supplies and Equipment

The following items, needed for the performance of the SPIFE Split Beta SPE Kit, must be ordered individually.

	Cat. No.
SPIFE 3000 Analyzer	1088
QuickScan 2000	1660
Electrophoresis Sample Handler	1341
Applicator Blade Weights	3387
Applicator Blades (for urine or CSF)	3450
Gel Block Remover	1115
SPE Normal	3424
SPE Abnormal	3425
REP Prep	3100
SPIFE Dispo Sample Cups (deep well)	3360
SPIFE Dispo Sample Cups (for Urine & CSF)	3369
SPIFE 2000/3000 Dispo Cup Tray	3370
SPIFE Urine/CSF Protein Accessory Kit	3427
SPIFE Urine IFE Alignment Tray	3380
Acid Violet Stain	551758

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