

SPIFE® 4000 Split Beta SPE Procedure

Cat. No. 2310

The SPIFE 4000 Split Beta SPE System is intended for the separation of serum or urine proteins and quantitation of serum proteins by agarose gel electrophoresis using the SPIFE 4000 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 Tiselius² 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, alpha₁, alpha₂, 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. 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.

REAGENT

1. SPIFE 4000 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 (Serum 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 entire contents of the vial of stain in 1 L of 5% acetic acid. Mix thoroughly for 30 minutes. Fill the appropriate 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 dissolved stain is stable six months when stored at 15 to 30°C.

Signs of Deterioration: The prepared 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 and mix well until completely dissolved. Pour the entire contents of the Destain Additive bottle into the prepared Destain.

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. Destain Additive

Ingredients: The product is a wetting agent.

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

Preparation for Use: Pour the entire contents of the Destain Additive bottle into the 11 L of prepared Citric Acid Destain.

Storage and Stability: The additive should be stored at 15 to 30°C and is stable until the expiration date indicated on the package.

Signs of Deterioration: The additive should be free of precipitate.

5. Acid Violet Stain (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 appropriate 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 4000 must be used to apply samples, electrophorese, stain, destain, dry and then scan the gels. Refer to the Operator's Manual for detailed instructions.

SPECIMEN COLLECTION AND HANDLING

Specimen: Fresh serum 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 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 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. Swirl samples to homogenize. Vigorous agitation may denature proteins. 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

Interfering Factors:

1. Hemolysis may cause false elevation in the alpha₂ 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 4000 Split Beta SPE Kit (Cat. No. 2310). Individual items are not available.

SPIFE 4000 Split Beta SPE Gels (10)
Acid Blue Stain (1 vial)
SPIFE 4000 Blotter C (10)
Citric Acid Destain (1 pkg)
SPIFE 4000 Split Beta SPE Applicator Blades (20)
Destain Additive (28 mL)

Material provided but not contained in the kit:

ITEM	CAT. NO.
SPIFE 4000 Analyzer	1620, 1621
Gel Block Remover	1115
SPE Normal Control	3424
SPE Abnormal Control	3425
SPIFE 4000 White Sample Trays	2315
SPIFE 4000 Cassettes	1630
SPIFE 4000 Maintenance Blotters	2307
SPIFE 4000 Applicator Blades (for urine)	2319
SPIFE 4000 Gel Staging Lid	2308

Acid Violet Stain (for urine) 552351
 SPIFE 4000 Replacement Electrodes 1625

Materials needed but not provided:

- 0.85% saline
- 5% acetic acid
- 10% acetic acid

STEP-BY-STEP METHOD

I. Sample Preparation

A. Serum

No specimen preparation is necessary. The SPIFE 4000 automatically dispenses samples into the sample tray.

B. Urine

Urine specimens cannot be run on the same gel with serum specimens. The applicator blade for urine (Cat No. 2319) is different and must be ordered separately. The running parameters are also different.

For urine specimen volumes measuring less than 500 µL, 40 µL must be hand-pipetted into the sample tray for application. If there is 500 µL or greater, the specimen can be transferred into a tube and automatically dispensed into the tray.

II. SPIFE 4000 Preparation

1. Stack the appropriate number of Disposable Sample Trays into the Sample Tray Holder (one tray per gel).
2. Place Split Beta SPE Applicator Blades (two per gel) in the Applicator Tray.
3. Fill the designated bottles with deionized water, 0.85% saline and destain.
4. Add prepared (acid blue or acid violet) stains to the appropriate stain bottle. The stains must be replaced after processing ten gels to avoid contamination.
5. Fill the DI Water Surfactant jar with deionized water, and replace the lid and tubing. Ensure that the ends of the tubing are below the water level.
6. Remove the antisera/water reservoir from the antisera station. Lift the cover, fill the "H₂O" well and replace the cover.
7. Turn on the SPIFE 4000. Wait about 3 minutes after turning on the lower unit. Click the SPIFE 4000 icon on the screen for the instrument to initialize.
8. Using the prompts, prime the sample handler and the surfactant delivery system according to the instructions in the Operator's Manual.
9. Carefully open one end of the pouch, remove the gel from the protective packaging and discard the overlay.
10. Using a SPIFE 4000 Blotter C, gently blot the entire gel. Discard the blotter.
11. Hold the gel so that the barcode is at the top. Place the gel into the cassette by holding the gel backing in one hand and gently bending the gel. Slide each end of the gel backing into the slots of the cassette to hold it in place. Align the cut out in the gel backing with the alignment pin in the cassette.
12. Ensure the gel blocks make good contact with the electrodes to prevent skewed patterns.
13. Place the cassette with the gel into the humidior and cover the topmost cassette with the Gel Staging Lid. Close the humidior lid to minimize gel dehydration.
14. Repeat Steps 9-13 for each gel needed.
15. Load patient sample test tubes into the carousel counterclockwise starting at the space numbered 1. Multiple carousels are loaded counterclockwise.

III. Electrophoresis Parameters

A. Serum

1. Using the instructions provided in the Operator's Manual, select -- **Test Type: Serum Proteins**
Test Name: Serum Proteins
 Check the programmed parameters for each of the following processes.

- [Sample Preparation](#)
- [Sample Application](#)
- [Electrophoresis](#)
- [Predry](#)
- [Stain](#)
- [Destain](#)
- [Dry](#)
- [Scan](#)

Sample Preparation	Volume (µL)	40
	Primary Wash Time (mm:ss)	00:02
	Primary Wash Cycles	0
	Samples per gel	28
	Application per sample	1
	Dilutions 1-28	Neat
Sample Application	Applicator Load Time (mm:ss)	00:30
	Applicator Load Speed	95
	Application Rows	2
	Row 1 Location (mm from gel edge):	45.0
	Apply Time (mm:ss)	00:10
	Apply Cycles	1
	Absorption Time (mm:ss)	00:45
	Inter-Gel Start Delay (mm:ss)	00:00
Electrophoresis	Voltage	400
	Minimum Current (mA)	10
	Maximum Current (mA)	100
	Temperature (°C)	21
	Time (hh:mm:ss)	00:06:00
Pre-Dry	Temperature (°C)	53
	Time (hh:mm:ss)	00:12:00
Stain	Stain Type	Acid Blue
	Absorption Time	00:04:00
Destain	Cycles	4
	Time (hh:mm:ss)	00:02:00
	Agitate	Yes
Dry	Temperature (°C)	60
	Time (hh:mm:ss)	00:13:00
Scan	Sequence	1-28
	Aperture Size	5
	Gain Mode	Auto
	Smooth	No
	Auto Interpretation	No
	Image Contrast	0.0
	Fraction Detection	
	Sensitivity	3.1
	Force Fractions	No
	Combine Split Beta	Yes

2. Proceed to Section IV. Electrophoresis.

B. Urine

1. Volume of 500 µL or Greater

- a. Pipette 500 µL or more into a test tube and place it in the sample carousel.
- b. Using the instructions provided in the Operator's Manual, select -- **Test Type: Urine Proteins**
Test Name: Urine Proteins

Check the programmed parameters for each of the following processes.

- [Sample Preparation](#)
- [Sample Application](#)
- [Electrophoresis](#)
- [Predry](#)
- [Stain](#)
- [Destain](#)
- [Dry](#)
- [Scan](#)

Sample Preparation	Volume (µL)	40
	Primary Wash Time (mm:ss)	00:02
	Primary Wash Cycles	1
	Samples per gel	28
	Application per sample	1
	Dilutions 1-28	Neat
Sample Application	Applicator Load Time (mm:ss)	00:30
	Applicator Load Speed	115
	Application Rows	2
	Row 1 Location (mm from gel edge):	45.0
	Apply Time (mm:ss)	01:00
	Apply Cycles	3
	Absorption Time (mm:ss)	00:00
	Inter-Gel Start Delay (mm:ss)	01:00
Electrophoresis	Voltage	400
	Minimum Current (mA)	0
	Maximum Current (mA)	100
	Temperature (°C)	19
	Time (hh:mm:ss)	00:07:00

Pre-Dry	Temperature (°C)	53
	Time (hh:mm:ss)	00:12:00
Stain	Type	Acid Violet
	Time	00:02:00
Destain	Cycles	4
	Time (hh:mm:ss)	00:02:00
	Agitate	No
Dry	Temperature (°C)	60
	Time (hh:mm:ss)	00:13:00
Scan	Sequence	1-28
	Aperture Size	5
	Gain Mode	Auto
	Smooth	No
	Auto Interpretation	No
	Image Contrast	0.0
	Fraction Detection	
	Sensitivity	5.0
	Force Fractions	No
	Combine Split Beta	No

c. Proceed to Section IV. Electrophoresis.

2. Urine Volumes less than 500 µL

- Pipette 40 µL of each urine specimen into the Sample Tray.
- Place the Sample Tray with specimens into the Sample Tray Holder.
- The SPIFE 4000 User Setup menu should be used to create "Add a Test" parameters for small volumes of urine which need to be manually pipetted. The test parameters will be the same as those given in Section III B.1 except that "Sample Preparation" will not be checked.
- Proceed with the testing according to steps in Section IV.

IV. Electrophoresis

- Click START on the screen and respond to the analyzer prompts. The analyzer will load samples when appropriate, apply samples, electrophorese, stain, destain, dry and scan the gels.
- After scanning, the cassette with the gel will be dropped into the cassette receptacle.
- Remove the cassette(s) from the receptacle. If gel storage is required, remove and discard the two gel blocks. Clean or wipe the non-gel side. If not, discard the used gels, blotters, applicator blades and sample trays as biohazardous waste.
- Cassettes and carbon electrodes should be washed and dried after each use with deionized water. Refer to the Operator's Manual for instructions.

Stability of End Product: The completed, dried SPIFE 4000 Split Beta 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. 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 50 normal specimens using the SPIFE 4000 Analyzer. These values are presented as a guideline.

Protein Fraction	% of Total Protein $\bar{X} \pm 2 S.D.$
Albumin	48.1 – 59.5
Alpha ₁	2.3 – 4.9
Alpha ₂	6.9 – 13.0
Beta	13.8 – 19.7
Gamma	10.1 – 21.9

Each laboratory should perform its own normal range study.

Variations of Expected Values⁵

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, alpha₂ and beta fractions, slightly increased alpha₁ 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, alpha₁, alpha₂, beta and gamma protein bands on SPIFE 4000 Split Beta SPE 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 alpha₁, followed by alpha₂ globulin, split beta and gamma globulins.

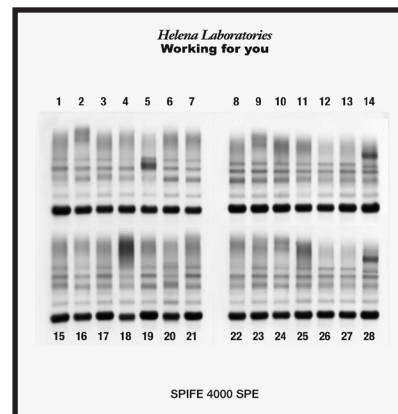


Figure 1: A SPIFE 4000 Split Beta SPE Gel showing relative position of the bands.

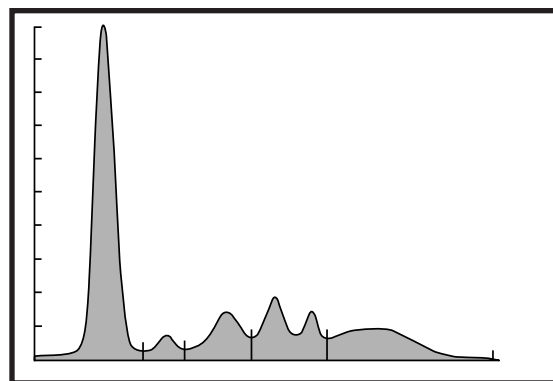


Figure 2: A scan of a SPIFE 4000 Split Beta SPE pattern.

Calculations of the Unknown

The SPIFE 4000 scanner 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 RESULTS⁵

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.

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 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 control, an abnormal control and a normal patient were run in replicate on a single gel with the following results: N = 28 each

Protein Fraction	Mean %	SD	CV
Albumin	56.4	1.1	2.0%
Alpha ₁	4.0	0.4	10.0%
Alpha ₂	10.0	0.7	7.0%

Beta	16.8	0.2	1.2%
Gamma	12.7	0.6	4.7%
Abnormal Control			
Protein Fraction	Mean %	SD	CV
Albumin	55.5	0.8	1.4%
Alpha ₁	3.4	0.2	5.9%
Alpha ₂	8.6	0.3	3.5%
Beta	13.6	0.4	2.9%
Gamma	18.9	0.6	3.2%
Normal Patient			
Protein Fraction	Mean %	SD	CV
Albumin	55.9	1.1	2.0%
Alpha ₁	3.4	0.3	8.8%
Alpha ₂	7.9	0.5	6.3%
Beta	15.5	0.4	2.6%
Gamma	17.4	0.5	2.9%

Between-Run: A normal control, an abnormal control and a normal patient sample were run alternately on eight gels.

Normal Control N = 80

Protein Fraction	Mean %	SD	CV
Albumin	58.3	1.0	1.7%
Alpha ₁	3.9	0.3	8.8%
Alpha ₂	9.3	0.4	4.7%
Beta	16.7	0.4	2.2%
Gamma	11.9	0.6	4.9%

Abnormal Control N = 72

Protein Fraction	Mean %	SD	CV
Albumin	54.0	1.0	1.9%
Alpha ₁	3.6	0.2	5.2%
Alpha ₂	8.8	0.3	3.8%
Beta	14.0	0.4	3.2%
Gamma	19.7	0.6	3.0%

Normal Patient N = 72

Protein Fraction	Mean %	SD	CV
Albumin	55.7	1.0	1.9%
Alpha ₁	3.4	0.3	7.4%
Alpha ₂	8.0	0.4	5.2%
Beta	15.5	0.4	2.7%
Gamma	17.4	0.6	3.6%

Correlation

Fifty normal and fifty abnormal serum samples were analyzed in duplicate using the SPIFE 3000 Split Beta SPE system and the SPIFE 4000 Split Beta SPE system.

N = 100

$$Y = 0.967X + 0.645$$

R = 0.999

X = SPIFE 3000 Split Beta SPE

Y = SPIFE 4000 Split Beta SPE

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SPIFE® 4000 SPLIT BETA SPE Kit

Cat. No. 2310

- SPIFE 4000 Split Beta SPE Gels (10)
- Acid Blue Stain (1 vial)
- SPIFE 4000 Blotter C (10)
- Citric Acid Destain (1 pkg)
- SPIFE 4000 Split Beta SPE Applicator Blades (20)
- Destain Additive (28 mL)

Other Supplies and Equipment

The following items, needed for the performance of the SPIFE 4000 Split Beta SPE Kit, must be ordered individually.

	Cat. No.
SPIFE 4000 Analyzer	1620, 1621
Gel Block Remover	1115
SPE Normal Control	3424
SPE Abnormal Control	3425
SPIFE 4000 White Sample Trays	2315
SPIFE 4000 Cassettes	1630
SPIFE 4000 Maintenance Blotters	2307
SPIFE 4000 Applicator Blades (for urine)	2319
SPIFE 4000 Gel Staging Lid	2308
Acid Violet Stain (for urine)	552351
SPIFE 4000 Replacement Electrodes	1625

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