

SPIFE® SPE Procedure

Helena  Laboratories

Cat. No. 3460, 3461, 3462

The SPIFE SPE System is intended for the separation of serum, cerebrospinal fluid (CSF) or urine proteins by agarose gel electrophoresis using the SPIFE, SPIFE 2000 or 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 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. 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.

REAGENT

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.

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 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®, SPIFE 2000 or 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 2000 (Cat. No. 1660). 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 or 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 alpha₂ 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)	
Modified Applicator Blade Assembly-20 Sample	

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

ITEM	Cat. No.
SPIFE Analyzer	1109
SPIFE Analyzer 2000	1130
SPIFE Analyzer 3000	1088
QuickScan 2000	1660
Gel Block Remover	1115
SPE Normal	3424
SPE Abnormal	3425
REP Prep	3100
Applicator Blade Weights	3387
SPIFE Dispo Cups (deep well)	3360
Disposable Sample Cups (for Urine/CSF)	3369
SPIFE 2000/3000 Dispo Cup Tray	3370
SPIFE/REP Dispo Cup Tray	1343
Applicator Blades (for Urine/CSF)	3450
SPIFE Urine/CSF Protein Accessory Kit	3427
SPIFE Urine IFE Alignment Tray	3380
Acid Violet Stain	552351

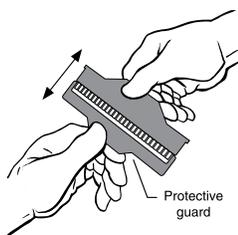
Materials needed but not provided:

- 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. Remove the protective guard from the blades by gently bending



2. Place the three Applicator Blades into the numbered vertical slots in the Applicator Assembly as given below.

Instrument	Slots
SPIFE	2,9,16
SPIFE 2000/3000	2,8,14

If using fewer Applicator Blades, place them into any two of the three slots noted above for the appropriate instrument.

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.

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.

3. Place an Applicator Weight on top of each Applicator Blade.
4. If using SPIFE, raise the applicator blades by flipping the toggle switch to the up position. Using the adjustment knob, set the Auto Applicator speed to 1.
5. 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.
6. Pipette the following amount of sample into the cups. Cover the tray until ready to use.

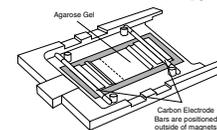
NOTE: Application of Urine and CSF samples cannot be done with the Applicator Blades or Cups packaged in the kit. Other Blades (Cat. No. 3450) and Cups (Cat. No. 3369) must be purchased.

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

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. 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. Close the chamber lid.
7. Press the **TEST SELECT/CONTINUE** button 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 appropriate Operator's Manual, set up the parameters as follows for the SPIFE, SPIFE 2000, or SPIFE 3000.

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.

A. SPIFE

Electrophoresis Unit

• Serum

- 1) Load Sample 00:30 21°C
Place applicator on sample tray (continue)
- 2) Apply Sample 00:30 21°C
Place applicator on chamber (continue)
- 3) Electrophoresis 6:00 21°C 650V
Remove applicator, close lid, (continue)
- 4) Dry *10:00 54°C
Remove gel blocks, (continue)
- 5) END OF TEST
No Prompt

• CSF or Urine

- 1) Load Sample 00:30 21°C
Place applicator on sample tray (continue)
- 2) Apply Sample 00:30 21°C
Place applicator on chamber (continue)
- 3) Load Sample 00:30 21°C
Place applicator on sample tray (continue)
- 4) Apply Sample 00:30 21°C
Place applicator on chamber (continue)
- 5) Load Sample 00:30 21°C
Place applicator on sample tray (continue)
- 6) Apply Sample 1:00 21°C
Place applicator on chamber (continue)
- 7) Absorb 1:00 21°C
No Prompt
- 8) Electrophoresis 6:00 21°C 650V
Remove applicator, close lid, (continue)
- 9) Dry *10:00 54°C
Remove gel blocks, (continue)
- 10) END OF TEST
No Prompt

If testing serum, place an Applicator Blade into the assembly so that the blade aligns with the serum cups. The blade can only be added after Step 4).

Stainer Unit

• Serum and CSF or Urine

- 1) Stain 4:00 Recirculate OFF
No Prompt
- 2) Destain 1:00 Recirculate ON
No Prompt
- 3) Destain 1:00 Recirculate ON
No Prompt
- 4) Destain 1:00 Recirculate ON
No Prompt
- 5) Dry *12:00 63°C
No Prompt

6) END OF TEST

No Prompt

1. 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. Open the chamber lid.
2. The Auto Applicator speed should be set at 1. Place the Auto Applicator Assembly onto the base/tray alignment pins. Lower the applicator tips down into the sample wells by flipping the toggle switch to the down position. Press **TEST SELECT/CONTINUE** to time sample loading.
3. After 30 seconds, lift the applicator tips out of the wells by flipping the toggle switch to the up position. Sample cups should be discarded as biohazardous waste.
4. Carefully lift the entire applicator assembly away from the Applicator Base and immediately place it onto the alignment pins located on the SPIFE electrophoresis chamber. Lower the applicator blades down onto the gel by flipping the toggle switch to the down position.
5. Close the chamber lid, and press the **TEST SELECT/CONTINUE** button. Sample application will be timed.
6. After sample application is complete, open the chamber lid and carefully lift the applicator blades by flipping the toggle switch to the up position.
7. For urines and CSFs, repeat Steps 2-6 two more times for a total of three applications.
8. Remove the Applicator Assembly from the electrophoresis chamber. (Discard the applicator blades as biohazardous waste.)
9. Close the chamber lid, and press the **TEST SELECT/CONTINUE** button to start electrophoresis. SPIFE will beep when electrophoresis is complete.

B. SPIFE 2000

Electrophoresis Unit

• Serum

- 1) No Prompt
Load Sample 1 00:30 21°C SPD.=1
- 2) No Prompt
Apply Sample 1 00:30 21°C SPD.=1
- 3) No Prompt
Electrophoresis 1 6:00 21°C 650 V
- 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 SPD.=1
- 2) No Prompt
Apply Sample 1 00:30 21°C SPD.=1
- 3) No Prompt
Load Sample 2 00:30 21°C SPD.=1
- 4) No Prompt
Apply Sample 2 00:30 21°C SPD.=1
- 5) To Continue, (continue)
Load Sample 3 00:30 21°C SPD.=1
- 6) No Prompt
Apply Sample 3 1:00 21°C SPD.=1

- 7) No Prompt
Absorb 1 1:00 21°C
- 8) No Prompt
Electrophoresis 1 6:00 21°C 650V
- 9) Remove gel blocks, (continue)
Dry 1 *10:00 54°C
- 10) No Prompt
END OF TEST

Stainer Unit

● **Serum, 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 CIR = OFF VALVE = 3
 - 2) No Prompt
Destain 1 1:00 CIR = ON VALVE = 2
 - 3) No Prompt
Destain 2 1:00 CIR = ON VALVE = 2
 - 4) No Prompt
Destain 3 1:00 CIR = 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 2000. Align the holes in the tray with the pins on the instrument. Close the chamber lid.
 2. With **SERUM/URINE PROTEIN** 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. The SPIFE 2000 will apply the samples, electro-phorese and beep when completed.
 3. Open the chamber lid and place the Modified Blade in place for serum application. Close the chamber lid. Press **TEST SELECT/CONTINUE**.

C. SPIFE 3000

Electrophoresis Unit

● **Serum**

- 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 6:00 21°C 650V 135mA
- 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 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) To Continue, (continue)
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 6:00 21°C 650V 135mA
- 9) Remove gel blocks, (continue)
Dry 1 *10:00 54°C
- 10) No Prompt
END OF TEST

Stainer Unit

● **Serum, 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. Close the chamber lid.
 2. With **SERUM/URINE PROTEIN** 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. The SPIFE 3000 will apply the samples, electro-phorese and beep when completed.
 3. Open the chamber lid and place the Modified Blade in place for serum application. Close the chamber lid. Press **TEST SELECT/CONTINUE**.

IV. VISUALIZATION

1. After electrophoresis is complete, use the Gel Block Remover to remove the gel blocks. Dispose of blades and cups as biohazardous waste. Place one electrode across 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 backing 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 **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. The instrument will stain, destain, and dry the gel.
7. 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 at 595 nm, agarose side down. 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 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 SPE System on 50 normal specimens using the SPIFE Analyzers. These values are presented as a guideline.

Protein Fraction	% of Total Protein
	$\bar{X} \pm 2 \text{ S.D.}$
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 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 with slightly increased alpha₁, 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, alpha₁, alpha₂, 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 alpha₁, followed by alpha₂ globulin, beta and gamma globulins.

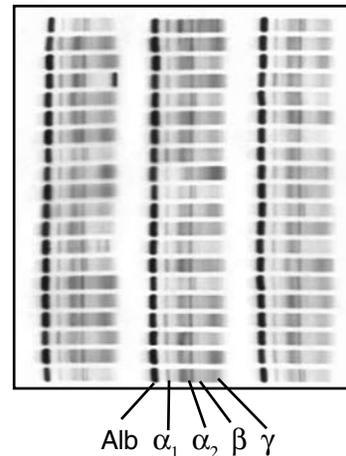


Figure 1: A SPIFE SPE-60 Gel showing relative position of the bands

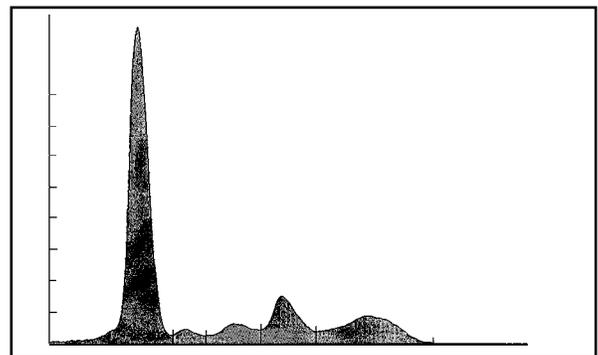


Figure 2: A scan of a SPIFE SPE pattern.

Calculations of the Unknown

The Helena QuickScan 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 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.

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 = 60

Protein Fraction	Mean %	SD	CV%
Albumin	52.6	1.3	2.5
Alpha ₁	3.5	0.2	5.7
Alpha ₂	12.6	0.4	3.4
Beta	15.8	0.5	3.3
Gamma	15.4	0.5	3.3

Between-Run: A pooled serum sample was analyzed 60 times on each of 6 gels. N = 360

Protein Fraction	Mean %	SD	CV%
Albumin	58.3	1.4	2.7
Alpha ₁	3.5	0.3	7.4
Alpha ₂	12.4	0.4	3.2
Beta	12.4	0.4	3.2
Gamma	15.5	0.6	3.9

CORRELATION

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

N	= 96	
Slope	= 0.972	Y = 0.972X + 0.560
Intercept	= 0.560	X = SPIFE SPE Vis
R	= 0.997	Y = SPIFE SPE

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SPIFE SPE System

Cat. No. 3460, 3461, 3462

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

Other Supplies and Equipment

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

	Cat. No.
SPIFE Analyzer	1109
SPIFE 2000 Analyzer	1130
SPIFE 3000 Analyzer	1088
QuickScan 2000	1660
SPIFE Gel Block Remover	1115
SPE-Normal	3424
SPE-Abnormal	3425
REP Prep	3100
Applicator Blade Weights	3387
SPIFE Dispo Cups (deep well)	3360
Disposable Sample Cups (for Urine/CSF)	3369
SPIFE 2000/3000 Dispo Cup Tray	3370
SPIFE/REP Dispo Cup Tray	1343
Applicator Blades (for Urine/CSF)	3450
SPIFE Urine/CSF Protein Accessory Kit	3427
SPIFE Urine IFE Alignment Tray	3380
Acid Violet Stain	552351

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