

SPIFE® Touch Split Beta SPE - 80, 100 Procedure

Cat. No. 3399, 3398

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 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 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. **The 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 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. **The stain must be replaced after processing 10 gels to avoid contamination.**

INSTRUMENTS

A SPIFE Touch Analyzer 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 is necessary, serum 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 one 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 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 Split Beta SPE - 80, 100 Kits. Individual items are not available.

Test Size	CAT. NO.
100 Samples	3398
80 Samples	3399

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 2000	1660
QuickScan Touch	1690
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 20-100 Dispo Cup Tray	3366
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 81 to 100 samples, remove five Disposable Applicator Blades from the packaging. If testing fewer samples, remove the appropriate number of Applicator Blades from the packaging.
2. Place five Applicator Blades into the numbered vertical slots 2, A, 9, 13 and 16 in the Applicator Assembly as given below. If using fewer Applicator Blades, place them into any of the 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 urine/CSF, 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 Cup strips into the Cup Tray. If testing less than 81 samples, pipette samples into the rows of cups that correspond with 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 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.

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/CSF (Template Application)

Specimens with insufficient volumes may be run using the SPIFE Urine/CSF 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 may 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: 1

Electrophoresis Prompt: None
Time: 6:00
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: 1

Electrophoresis Prompt: None
Time: 6:00
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: 1

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: 1

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: 1

Electrophoresis

Prompt: None
Time: 6:00
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: 1

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: 1

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: 1

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: 1

Electrophoresis

Prompt: None
Time: 6:00
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: 6:00
Temperature: 21°C
Voltage: 650 V
mA: 135 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 1 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 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. Dispose of blades and cups as biohazardous waste.
3. Close the chamber lid and press the **CONTINUE** button to dry. 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. Dispose of blades and cups as biohazardous waste.
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 20 available slits on the Urine/CSF Template.
3. Close the chamber lid, and press the **CONTINUE** button for the electrophoresis chamber. 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 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. 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

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

Quantitative Evaluation: Scan the serum samples on 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 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 SPIFE Split Beta SPE and the QuickGel Split Beta SPE Systems 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 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.

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 Split Beta SPE-100 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, beta and gamma globulins.

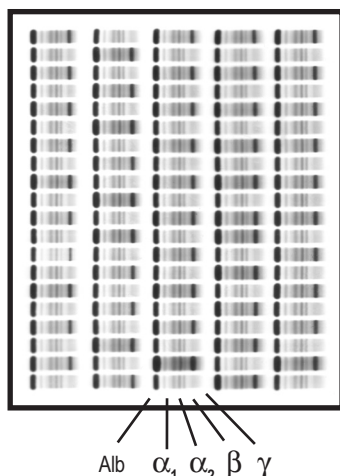


Figure 1: A SPIFE Split Beta SPE-100 Gel showing relative position of the bands.

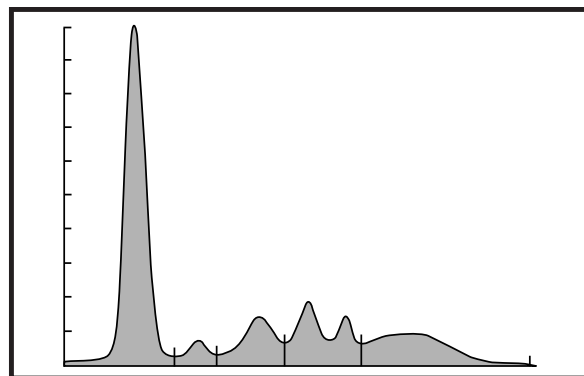


Figure 2: A scan of a SPIFE Split Beta SPE pattern.

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 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, plasmacell dyscrasias, pregnancy and genetic deficiencies.

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 on a single gel with the following results:

Normal Control (n = 34)

Protein Fraction	Mean %	SD	CV
Albumin	53.7	0.8	1.5%
Alpha ₁	4.0	0.2	4.3%
Alpha ₂	9.8	0.3	2.8%
Beta	16.9	0.2	1.4%
Gamma	15.6	0.5	2.9%

Abnormal Control (n = 33)

Protein Fraction	Mean %	SD	CV
Albumin	47.3	0.8	1.6%
Alpha ₁	3.5	0.1	3.6%
Alpha ₂	9.0	0.2	2.7%
Beta	13.0	0.2	1.8%
Gamma	27.2	0.4	1.4%

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

Normal Control (n = 304)

Protein Fraction	Mean %	SD	CV
Albumin	54.4	1.1	2.0%
Alpha ₁	3.9	0.2	6.0%
Alpha ₂	9.6	0.3	3.6%
Beta	16.6	0.4	2.7%
Gamma	15.5	0.5	3.4%

Abnormal Control (n = 292)

<u>Protein Fraction</u>	<u>Mean %</u>	<u>SD</u>	<u>CV</u>
Albumin	47.7	0.8	1.8%
Alpha ₁	3.5	0.2	5.1%
Alpha ₂	8.9	0.3	2.8%
Beta	12.8	0.3	2.3%
Gamma	27.1	0.4	1.5%

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 = 1.0043X - 0.083

R = 0.9999

X = SPIFE Split Beta SPE

Y = SPIFE Touch Split Beta SPE

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