

QuickGel® Touch SPE Procedure

Cat. No. 3405

The QuickGel Touch SPE method is intended for the separation of serum, cerebrospinal fluid (CSF) or urine proteins by agarose gel electrophoresis using the SPIFE Touch system. Cat. No. 3405 - for use with SPIFE Touch.

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 QuickGel Touch SPE procedure, 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. Quick Gel 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. 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 L of 10% acetic acid and mix thoroughly.

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.

INSTRUMENTS

A SPIFE Touch must be used to electrophorese, stain, destain and then dry the gels. The gels may be scanned on the QuickScan Touch/2000 (Cat. No. 1690/1660) or a separate densitometer. Refer to the appropriate 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.

Specimen Storage: If storage of serum 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 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 Specimen 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
100-100	50x
300-300	25x
600-600	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 FOR SPIFE TOUCH

Materials provided: The following materials needed for the procedure are contained in the QuickGel SPE Kit (Cat. No. 3405). Individual items are not available.

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

Material provided but not contained in the kit:

Item	Cat. No.
SPIFE Touch	1068
QuickScan Touch	1690
QuickScan 2000	1660
Applicator Blade Weights	3387
Gel Block Remover	1115
SPE Normal Control	3424
SPE Abnormal Control	3425
REP Prep	3100
Disposable Sample Cups (Shallow Wells)	3369
QuickGel Dispo Cup Tray	3353
SPIFE QuickGel Electrode	1111
SPIFE QuickGel Holder	3358
SPIFE QuickGel Chamber Alignment Guide	86541003
QuickGel Accessory Kit (Templates)	3426
Acid Violet Stain	552351

Materials needed but not provided:

5% acetic acid
0.85% saline

STEP-BY-STEP METHOD

I. Chamber Preparation

1. The SPIFE QuickGel Chamber Alignment Guide must be used to mark the location for gel placement. It is recommended that the markings be placed directly on the copper floor under the contact sheet.
2. Remove the contact sheet and clean the chamber floor according to instructions in the Operator's Manual.
3. Place the round hole in the guide over the left chamber pin and the obround hole over the right pin.
4. Using an indelible marker, outline the square open area onto the copper floor. Allow marking to dry and apply another contact sheet.

II. Gel Preparation for Blade Application

1. Remove one Disposable Applicator Blade from the packaging.
2. Place the Applicator Blade into the vertical slot numbered 6 in the Applicator Assembly. If using two Applicator Blades, place them into the vertical slots numbered 6 and 12. When testing serum with urine/CSF samples, serum application is made after the third urine or CSF application. Therefore the blade for serum application is not added until after the third urine/CSF application.

NOTE: The Applicator Blade 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)**”. If testing serum with urine/CSF, follow 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 Applicator Blade. When placing the weight on the blades, position the weight with the thick side to the right.
4. Slide the Disposable Sample Cups into the top row numbered 1 to 10 of the appropriate cup tray. If testing more than 10 samples, place cups into both rows.
5. Pipette 15 µL of control or serum or 20 µL of urine or CSF into Disposable Sample Cups (Cat. No. 3369 for both) numbered 1 to 5 and 6 to 10. If testing more than 10 samples, pipette sample into cups 11 to 15 and 16 to 20. Cover the tray until ready to use.
Specimens with insufficient volumes may be run using the QuickGel Accessory Kit (Cat. No. 3426).
6. Carefully cut open the end of the gel pouch. Remove one gel from the protective packaging. Fold and tape the open end of the pouch to prevent drying of the gel. Remove the gel from the plastic mold and discard the mold.
7. Place a QuickGel Blotter C on the gel with the longer edge parallel with the gel blocks. Gently blot the entire gel using slight fingertip pressure on the blotter and remove the blotter.
8. Dispense approximately 1 mL of REP Prep onto the left side of the electrophoresis chamber.
9. Place the gel over the REP Prep inside the rectangle on the chamber floor. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right side. 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 the gel remains in place and that no bubbles remain under the gel.
10. Clean the QuickGel Electrodes with deionized water before and after each use. Wipe with a lint-free tissue.
11. Place a QuickGel Electrode on the outside ledge of each gel block inside the magnetic posts. Improper contact of the electrodes and the gel blocks may cause skewed patterns. Close the chamber lid.
12. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. To check parameters, select the test, press **SETUP** and proceed to Step IV. Once parameters have been verified proceed to Step IV.A if running serum only or urine/CSF only or Step IV.B if running serum and CSF/urine.

III. Template Preparation

Template application may be used for testing CSF or urine specimens which have insufficient volumes for blade application.

1. Carefully open one end of the pouch and remove one gel from the protective packaging. Reseal the pouch with tape to prevent drying of the gel. Remove the gel from the plastic mold and discard the mold.
2. Place a QuickGel Blotter C on the gel with the longer edge parallel with the gel blocks. Gently blot the entire gel using slight fingertip pressure on the blotter and remove the blotter.
3. Dispense about 1 mL of REP Prep onto the left side of the marked area of the chamber.
4. Place the gel over the REP Prep inside the rectangle on the chamber floor. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right side. Use lint-free tissue to wipe around the edges of the gel backing to remove excess REP Prep. Make sure the gel remains in place and that no bubbles remain under the gel.
5. Depending on the number of samples tested, place one or two templates across the gel aligning the slits with the marks on the gel backing.
6. Apply fingertip pressure to the template, making sure there are no bubbles under it. **NOTE:** If wearing rubber gloves to perform this step, place a QuickGel Blotter A over the template and then apply fingertip pressure to the template. Remove the blotter. Powder from the gloves can produce gel artifacts.
7. Clean the electrodes with deionized water before and after each use. Wipe with a lint-free tissue.
8. Place a carbon electrode on the outside ledge of each gel block inside the magnetic posts. Improper contact of the electrodes and the gel blocks may cause skewed patterns. Close the chamber lid.
9. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. To check parameters, select the test, press **SETUP** and proceed to Step IV. Once parameters have been verified proceed to Step IV.C.

IV. Parameters

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

* **Due to variation in environmental conditions, a Dry time of 10 minutes is recommended, but a range of 10 to 15 minutes is acceptable.**

** **Due to variation in environmental conditions, a template application time of 10 minutes is recommended but a range of 7-10 minutes is acceptable.**

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

SEPARATOR UNIT

• Serum and Urine/CSF (Blade Application)

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: 6:30
Temperature: 21°C
Voltage: 375 V
mA: 80 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: 6:30
Temperature: 21°C
Voltage: 375 V
mA: 80 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: 6:30
Temperature: 21°C
Voltage: 375 V
mA: 80 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: 6:30
Temperature: 21°C
Voltage: 375 V
mA: 80 mA

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

End

STAINER UNIT

• **Serum, CSF and Urine (Both Application Methods)**

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: 10:00*
Temperature: 63°C

End

A. Serum or Urine/CSF (Blade Application)

1. Open the chamber lid and place the Cup Tray with samples on the SPIFE Touch. Align the holes in the tray with the pins on the instrument. Close the chamber lid.
2. 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, electrophoresis and beep when completed. Proceed to Step V.

B. Serum and Urine/CSF (Blade Application)

1. Open the chamber lid and place the Cup Tray with samples on the SPIFE Touch. Align the holes in the tray with the pins on the instrument. Close the chamber lid.
2. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** and choose an option 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 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 urine/CSF blades. Close the chamber lid and press **CONTINUE**. The instrument will apply and continue. Proceed to Step V.

C. Urine/CSF (Template Application)

1. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** and choose an operation to proceed.
2. Open the chamber lid and apply urine and/or CSF by placing 3 µL of each sample onto one of the ten available slits on the Sample Template.
3. Close the chamber lid and press the **CONTINUE** button for the electrophoresis chamber. Sample application will be timed for 10 minutes.
4. Open the chamber lid and gently blot the template with a QuickGel Blotter A and carefully remove the blotter and template. Dispose of templates as biohazardous waste.
5. Close the chamber lid and press the **CONTINUE** button to start electrophoresis. The SPIFE Touch will beep when electrophoresis is complete.

V. Visualization

1. After electrophoresis is complete, open the chamber lid and use the Gel Block Remover to remove the gel blocks. Dispose of blades and cups as biohazardous waste. Replace the electrodes on each end of the gel to prevent curling during drying.
2. Close the chamber lid and press the **CONTINUE** button to dry the gel.
3. After the gel has been dried, carefully remove the gel from the electrophoresis chamber.
4. Remove the SPIFE QuickGel Holder from the stainer chamber. While holding the gel agarose side down, slide one side of the gel backing under one of the metal bars. Bend the gel backing so that the gel is bowed, and slip the other side under the other metal bar. The two small notches in the backing must fit over the small pins to secure the gel to the holder.
5. Place the SPIFE QuickGel Holder with the attached gel facing backwards into the stainer chamber.
6. 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.
7. When the process is completed, the instrument will beep. Carefully remove the SPIFE QuickGel Holder from the stainer because the metal piece on the holder will be hot. Scan the bands in a densitometer or the QuickScan Touch/2000.

EVALUATION OF THE PROTEIN BANDS

Qualitative Evaluation: The urine and CSF samples run on the QuickGel SPE Gel can only be visually inspected for the presence of the bands.

Quantitative Evaluation: Scan the samples on the QuickGel 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, QuickGel 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 range presented was established on 50 normal specimens using the QuickGel SPE System on the SPIFE Touch Analyzer and the SPIFE 3000. These values are presented as a guideline. Each laboratory should perform its own normal range study.

Protein Fraction	% of Total Protein $\bar{x} \pm 2 \text{ S.D.}$
Albumin	46.5 - 58.8
Alpha ₁	1.8 - 4.6
Alpha ₂	9.2 - 16.5
Beta	11.2 - 18.8
Gamma	9.1 - 23.5

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 QuickGel 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, beta and gamma globulins.

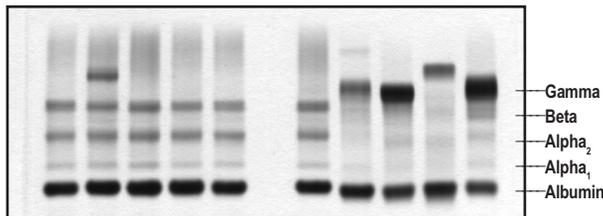


Figure 1: A QuickGel SPE Gel showing relative position of the bands.

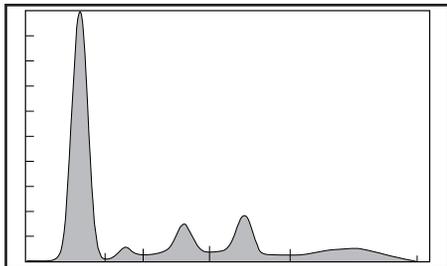


Figure 2: A scan of a QuickGel SPE pattern.

Calculations of the Unknown

The Helena QuickScan Touch/2000 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 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 serum to obtain optimal resolution and reproducible results. Noncompliance with the recommended procedure may affect the results.

PERFORMANCE CHARACTERISTICS FOR SPIFE TOUCH

PRECISION

Within Run: A normal and an abnormal control were run alternately on a single gel with the following results: n = 7

Normal Control			
Protein Fraction	Mean %	SD	CV
Albumin	53.3	0.8	1.4%
Alpha ₁	3.6	0.1	3.8%
Alpha ₂	12.7	0.2	1.5%
Beta	14.8	0.4	3.0%
Gamma	15.6	0.5	3.2%

Abnormal Control			
Protein Fraction	Mean %	SD	CV
Albumin	48.6	0.4	0.8%
Alpha ₁	3.2	0.2	5.7%
Alpha ₂	11.0	0.3	2.5%
Beta	10.5	0.3	2.7%
Gamma	26.8	0.3	1.1%

Between-Run: A normal control and an abnormal control were run in replicate on three SPIFE Touch instruments with the following results: n = 63

Normal Control			
Protein Fraction	Mean %	SD	CV
Albumin	53.7	1.0	1.9%
Alpha ₁	3.6	0.2	6.2%
Alpha ₂	12.6	0.4	2.9%
Beta	14.6	0.5	3.1%
Gamma	15.6	0.6	4.0%

Abnormal Control			
Protein Fraction	Mean %	SD	CV
Albumin	48.6	0.8	1.7%
Alpha ₁	3.1	0.2	5.9%
Alpha ₂	10.7	0.3	2.9%
Beta	10.8	0.4	3.5%
Gamma	26.8	0.4	1.6%

CORRELATION

28 normal and abnormal serum samples, as well as normal and abnormal controls, were analyzed using the SPIFE SPE system and the QuickGel SPE system.

$$n = 30$$

$$Y = 1.0201X + 0.4027$$

$$R = 0.9996$$

$$X = \text{QuickGel SPE Gel on SPIFE 3000}$$

$$Y = \text{QuickGel SPE Gel on SPIFE Touch}$$

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