

REP® SPE-8 Template Procedure

Cat. No. 3172

Helena  Laboratories

The Helena REP SPE Template System is intended for the separation and quantitation of serum proteins by agarose gel electrophoresis.

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, α_1 , α_2 , 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 Helena REP 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 acid blue solution.

REAGENT

1. REP SPE-8 Template Gel

Ingredients: Each gel contains agarose in a tris/sodium barbital/MOPS buffer with salicylic acid, citric acid, stabilizers and thimerosal as a preservative.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY.

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 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.
-- IRRITANT -- 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.

INSTRUMENT

A Rapid ElectroPhoresis Analyzer (REP) must be used to electrophorese, dry and scan the gels. The gel may be stained manually or with the REP Gel Processor. Refer to the appropriate Operator's Manual for detailed instructions.

SPECIMEN COLLECTION AND HANDLING

Specimen: Fresh serum 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. Serum or plasma specimens must be diluted before testing.

Interfering Factors:

1. Hemolysis may cause false elevation in the α_2 and beta fractions.
2. Inaccurate results may be obtained on specimens left uncovered, due to evaporation.

Storage and Stability: Fresh serum or plasma is the specimen of choice. If storage is necessary, samples may be stored covered at 15 to 30°C for 4 days or 2 to 6°C for 2 weeks, or -20°C for 6 months.⁶

PROCEDURE

Materials provided: The following materials needed for the procedure are contained in the REP SPE Template Kit. Individual items are not available.

REP SPE-8 Template Gels (10)
Acid Blue Stain (1 vial)
REP Blotter A (10)
REP Sample Cups (80 cups)
REP Templates (20)
REP Blotter A-Plus (20)
REP Blotter C (10)

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

ITEM	CAT. NO.
Kemtrol-Normal	7024
Kemtrol-Abnormal	7025

REP Gel Staining Dish (10)	1362
SUREprep	1574
REP Prep	3100
REP Gel Processor	1357
REP Alignment Tray	

- Slide the lid into place until it snaps.
- Using the instructions provided in the appropriate Operator's Manual, set up parameters on the screen as follows:

REP

Sample Location [Row]	AB
Sample Application Time	1 sec
Sample Application Volume	3.0 µL
Sample Absorption Time	1:30 mm:ss
Needle Wash Cycles	2
Needle Blot Time	1 sec
Electrophoresis Time	6:00 mm:ss
Electrophoresis Voltage	650 volts
Electrophoresis Current	0 mA
Electrophoresis Temperature	21°C
Air Dry Time	0:00 mm:ss
Reagent Pour Time	0 sec
Incubation Time	0:00 mm:ss
Incubation Temperature	0°C
Dry Time	8:00 mm:ss
Dry Temperature	54°C
Standby Temperature	21°C

Depress the F1 key, and the REP unit will automatically apply the samples to the gel.

IMPORTANT: Proper sample tip alignment is critical to this procedure. At the completion of each row's sample application, there is a one-minute sample tip wash cycle. During the wash cycle, ensure that all 4 samples were applied directly into the template slit. If a sample was applied to the side of the slit, use a pipettor tip to pull the sample across and into the slit. Care must be taken to avoid contamination or carryover. **Caution must be used here because the gantry is still going to move.**

- Following sample application and absorption time, remove the templates as follows. When Sample Absorption Time displays 1 minute, open the chamber lid, blot the template on row A with a Blotter A-Plus, then remove the template. At the end of the absorption time, blot the template on row B with a Blotter A-Plus, then remove the template. Close the chamber lid and electro-phoresis will begin.

B. Visualization of the Protein Bands

Manual Staining

- At the end of the electrophoresis and drying period, remove the gel from the chamber and place it on a blotter, agarose side up. Using a blade or straight edge, remove the two gel blocks from the gel completely and discard the gel blocks. The gel blocks interfere with staining. The gel will not destain properly if it is not completely dry. Gels are dry when a uniform dull sheen covers the gel.
- Fill one container of the Staining Set with prepared stain. Fill another container with 5% acetic acid.
- Place the gel into the Staining Dish containing the prepared stain for 4 minutes.
- Remove the gel from the stain and allow it to drain on a blotter. Destain the gel in two (2) consecutive washes of destain solution. Use a gentle alternately rocking and swirling technique. Allow the gel to remain in each wash for 1 minute. The gel background should be completely clear.

Materials needed but not provided:

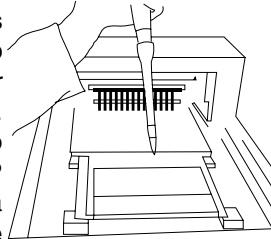
Glacial Acetic Acid - 5% solution - Mix 1900 mL deionized water and 100 mL glacial acetic acid.
0.85% Saline Solution

STEP-BY-STEP METHOD

A. Sample Application

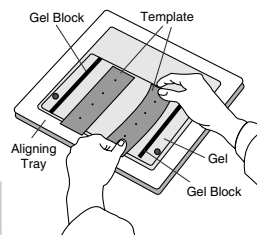
- Dilute each patient sample and control 1:4 with 0.85% Saline Solution (1 part sample + 3 parts 0.85% Saline Solution).

- Place 4 sample cups into wells 2, 3, 4 and 5; and 4 cups into wells 17, 18, 19 and 20 (color code with a yellow stripe). Place 50-75 µL of sample into each sample cup. Place REP Blotter A on sample tray in area adjacent to sample cups. Place approximately 4 mL of SUREprep into outside washwell of sample tray. Place approximately 4 mL of water into inside washwell of sample tray.

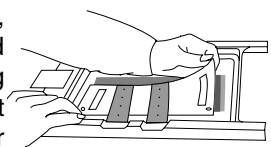


- Dispense approximately 1 mL of REP Prep onto left side of REP chamber.

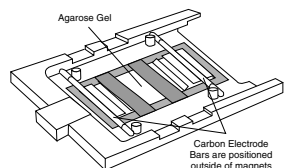
- Remove the gel from the protective packaging and discard overlay. Carefully place the gel on the Alignment Tray. Both areas of application are located between the small alignment pins on the tray. Using a REP Blotter C, gently blot the entire gel using slight fingertip pressure on the blotter. Remove the blotter and place 2 templates on the gel aligning the application slits with the pins on the sides of the Alignment Tray. The templates have been marked with a hole in one corner. Place the marked corner in lower left position. Apply slight fingertip pressure to each template, making sure there are no air bubbles under them.



- Carefully remove the gel from the tray, and place the left edge of the gel over REP Prep aligning the round hole on the left pin. 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 paper towel or absorbent paper to wipe around the edges of the gel backing, especially next to electrode posts to remove excess REP Prep. Make sure that the gel lays flat and that no bubbles remain under the gel.



- Before and after use, clean electrodes with deionized water and wipe with lint-free tissue.
- Place a carbon electrode on the outside ledge of each gel block outside the magnetic posts.



Between Run precision was evaluated with a patient serum specimen used in replicate on 5 gels. (N = 20)

Protein Fraction	Mean	SD	CV
Albumin	46.0%	1.8	3.9%
Alpha ₁	3.6%	0.4	9.9%
Alpha ₂	14.7%	0.8	5.3%
Beta	20.2%	0.7	3.5%
Gamma	15.4%	0.8	4.9%

CORRELATION

A correlation study of this method to the reference method yielded an excellent linear regression equation.

N = 58
 Y = 0.951 X + 0.950
 r = 0.992
 X = SPIFE SPE Vis
 Y = REP SPE-8 Template (Acid Blue)

BIBLIOGRAPHY

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5. Ritzmann, S.E., ed., Protein Abnormalities Vol I: Physiology of Immunoglobulins Diagnostic and Clinical Aspects, Allen R. Liss, Inc., New York, 1982.
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REP SPE TEMPLATE SYSTEM

REP SPE-8 TEMPLATE KIT	3172
REP SPE-8 Template Gels (10)	
Acid Blue Stain (1 vial)	
REP Blotter A (10)	
REP Sample Cups (80 cups)	
REP Templates (20)	
REP Blotter A-Plus (20)	
REP Blotter C (10)	

Other Supplies and Equipment

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

	Cat. No.
Rapid ElectroPhoresis (REP) Analyzer	1352
Kemtrol-Normal	7024
Kemtrol-Abnormal	7025
REP Prep Solution	3100
SUREprep	1574
REP Gel Staining Dish (10)	1362
REP Gel Processor	1357
REP Alignment Tray	

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REP Patent No. 4,810,348, 4,890,247, 4,909,920