**HELENA PROTEIN C ANTIGEN ROCKET EIA SYSTEM**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Protein C Antigen Rocket EIA Kit</th>
<th>Protein C Antigen Rocket Plates (5 plates/box)</th>
<th>Rocket Antigen</th>
<th>Report Form (1 form/box)</th>
<th>Other Supplies and Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>5357</td>
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</tbody>
</table>

**PERFORMANCE CHARACTERISTICS**

**Precision Studies**
- **Within-Run:** A control plasma was tested in replicate on one plate with the following results.
  - n = 26
  - X = 85.2% SD = 6.47 CV% = 8.0
- **Run-to-Run:** A control plasma was tested in replicate on four different plates giving the following data.
  - n = 8
  - X = 91.8% SD = 2.83 CV% = 3.0

**Correlation Studies**
Correlation studies were done on forty-three (43) normal and abnormal patient samples with the Helena Protein C method and the reference Protein C method. The study yielded an excellent linear regression equation and correlation coefficient of r = 0.937 Y = Reference Protein C method

**Bibliography**

**Protein C Antigen Rocket EIA Method**

**BIBLIOGRAPHY**

**For Sales, Technical and Order Information, and Service Assistance, call 800-231-5663 toll free.**

**Reagents**
- **Protein C Antigen Rocket Plates (Cat. No. 5357)**
- **Protein C Antigen Rocket EIA System Kit**
- **Coagulation S.A.R.P. (10 x 1.0 mL)**
- **S.A.C. -1 (10 x 1.0 mL)**
- **Titan GEL Chamber**
- **Microdispenser and Tubes (10 uL)**
- **Development Wells**
- **Slab Dish**

**Ingredients:**
- Each plate contains sheep or goat anti-Protein C antigen by Laurell rocket electrophoresis.1, 2
- The procedure is intended for the quantitative determination of plasma protein C antigen by Laurell rocket electrophoresis.3

**Summary:** Protein C is a vitamin K dependent plasma protein that functions as a regulator of fibrin formation. In its activated form, it inhibits thrombin formation by the inactivation of activated factors Va and VIIIa. 4 A deficiency of protein C constitutes a thrombotic risk factor5, 6 of which superficial thrombophlebitis is the most common clinical feature.7 The virtual absence of plasma protein C has led to fatal thrombosis in neonates.8

**Principles:** The Protein C Rocket EIA Procedure is performed in an agarose gel medium containing an antisem specific for protein C. After the plasma specimens are applied to the wells in the agarose, electrophoresis is used to migrate the proteins into the antibody field. A rocket-shaped precipitin pattern forms along the axis of migration. The length of this rocket pattern is proportional to the antigen concentration.

**References:**
Add nine parts whole blood to one part sodium citrate solution. Centrifuge the blood sample immediately after collection. Place the plasma, at 1000 × g, for 10 minutes. Store the plasma at 2 to 8°C until testing is performed. Plasma stored at 2 to 8°C must be tested within four hours after sample collection. Plasma is stable at -20°C for one month. Plastic tubes must be used for storage and testing.

**PROCEDURE**

**Materials Provided:**

- Cat.No.
- Protein C Antigen Rocket Plates (5 plates/box)
- Protein C Antibody Rocket Plates (5 plates/box)
- S.A.R.P. (10 x 1.0 mL)
- Tris-tricine Buffer (5 pkgs/box)
- Sponge Wicks (2/pkg)
- Helena Rocket Ruler
- Microdispenser and Tubes (10 µL)
- Development Weight
- Staining Dish

**SUMMARY OF CONDITIONS**

- Buffer ............................................................... Tris-tricine diluted to 1000 mL
- Sample Volume ........................................ 10 µL (15 µL if desired)
- Electrophoresis Time ...................................... 3 hours
- Amperage ......................................................... 16 mA/plate constant current
- Staining Time ................................................ 20 minutes

**STEP-BY-STEP METHOD**

**A. Preparation of Standards and Test Plasma**

1. Reconstitute one vial of S.A.R.P. with 1.0 mL deionized or distilled water. Make dilutions for preparation of the Standard Curve as follows:

<table>
<thead>
<tr>
<th>Percent</th>
<th>Activity Dilution</th>
<th>Parts</th>
<th>Parts</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>S.A.R.P.</td>
<td>100%</td>
<td>S.A.R.P. 0.85% Saline</td>
</tr>
<tr>
<td>50%</td>
<td>1:2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>25%</td>
<td>1:4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>12.5%</td>
<td>1:8</td>
<td>1</td>
<td>7</td>
</tr>
</tbody>
</table>

2. Dilute each patient sample and control with 0.85% saline. Prepare a 1:2 dilution (1 part patient sample and 1 part saline) and a 1:4 dilution (1 part patient plasma and 3 parts saline). Additional dilutions may be necessary depending on the patient history. Suspected abnormal samples may need to be further diluted.

**B. Preparation of Chamber**

1. Pour 65 mL Tris-tricine buffer into each inner section of the TITAN GEL chamber.

2. Prepare the Tris-tricine buffer (5 pkgs/box) and store it in a cool place.

3. Place the plate from the Destaining Solution and rinse it briefly in deionized water.

4. Dry the plates at 37°C for 5 minutes or at room temperature until dry.

5. Destain the plate by placing it in Destaining Solution until the rockets can be distinguished easily. The background will still be a bluish purple color. If a clearer background is desired, the plate can be transferred to fresh destain after 30 minutes and left in the destain overnight.

6. Remove the plate from the Destaining Solution and rinse it briefly in deionized water.

7. Dry the plates at 37°C for 5 minutes or at room temperature until dry.

8. Destain the plate by placing it in Destaining Solution until the rockets can be distinguished easily. The background will still be a bluish purple color. If a clearer background is desired, the plate can be transferred to fresh destain after 30 minutes and left in the destain overnight.

9. Remove the plate from the Destaining Solution and rinse it briefly in deionized water.

10. Dry the plates at 37°C for 5 minutes or at room temperature until dry.

**F. Measurements and Calculations**

1. Place the plate on the lightbox or on a piece of white paper for easier viewing of the rockets. Mark the apex of each rocket peak with marker.

2. Use the Helena Rocket Ruler, measure the length of each peak in millimeters. The peak is measured from the top of each well to the apex of the rocket.

3. Plot the values of the standard curve versus each rocket height on the Rocket Antigen Report Form or on cycle semi-logarithmic paper. Draw the line of best fit for the four points.

4. See Figures 1 and 2 for an example of a completed Rocket Plate and a standard curve drawn on a Rocket Antigen Report Form.

5. The patient value from curve = 30% Dilution factor = 2 S.A.R.P. assigned value = 98% Actual Patient Factor

6. Protein C Antigen = 30% x 2 x 0.98 = 58.8% Protein C Antigen = 30% x 2 x 0.98 = 58.8%

**Q. Quality Control**

A control material of known concentration, such as S.A.C.-1 (Cat. No. 5301) should be used on each run to verify all phases of the procedure and should be used on each gel. Refer to the package insert provided with the control for reconstitution instructions and assay values. If the control does not perform as expected, the patient results should be considered invalid or suspect.

**REFERENCE VALUES**

Protein C antigen values are usually expressed in relative percentages compared to a pooled normal plasma standard. The expected normal range for protein C antigen run by rocket electrophoresis has been reported at 95-129% with newborns showing less than 50% levels. L. B. Bertina et al., 1 reported a range of 65-145% in healthy individuals with diminished levels found following anticoagulant therapy. There is apparently no difference in protein C antigen levels between healthy males and females. 2 Helena tested 39 plasmas of presumed healthy men and women. The results were as follows:

- Male: 101% ± 24
- Female: 53 - 149%

Each laboratory should establish its own normal range for this procedure.

**LIMITATIONS**

Most patients with congenital protein C deficiency show diminished levels of both immunologic and functional activity, the so-called type I deficiency. 3 However, the finding of several patients with normal levels of protein C antigen and diminished functional protein C activity, type II deficiency, makes the diagnosis more difficult. The Helena procedure will detect only the type I deficiencies.

**TROUBLESHOOTING**

1. When applying the samples to the plate wells, do not allow the pipette tip to touch the sides of the wells.

2. Do not overdry plate. Overdrying will cause the plate to peel.

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**Figure 1:** Rocket patterns on a Protein C Antigen Rocket Plate. The lengths of the rockets (in millimeters) of the standard dilutions are used to prepare the standard curve. Patient results are read from the curve.

**Figure 2:** Representative standard curve prepared with S.A.R.P. on 3 cycle semi-logarithmic paper.

5. Read the patient values from the standard curve and multiply each by the appropriate dilution factor. If S.A.R.P. is used to prepare the standard curve, the factor must be multiplied by the assigned Protein C Antigen value of the appropriate lot of S.A.R.P. as well as the dilution factor.

Example:

<table>
<thead>
<tr>
<th>Patient value from curve = 30%</th>
<th>Dilution factor = 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.A.R.P. assigned value = 98%</td>
<td>Actual Patient Factor</td>
</tr>
</tbody>
</table>

Protein C Antigen = 30% x 2 x 0.98 = 58.8%
Add nine parts whole blood to one part sodium citrate solution. Centrifuge the blood sample immediately after collection at 1000 g for 10 minutes. Store the plasma at 2 to 8°C until testing is performed. Plasma stored at 2 to 8°C must be tested within four hours after sample collection. Plasma is stable at -20°C for one month. Plastic tubes must be used for storage and testing.

**PROCEDURE**

**Materials provided:** Cat.No. Protein C Antigen Rocket Plates (5 plates/box) 5357

**Rocket Antigen Report Form (1 form/box)**

**Helena Rocket Ruler**

**Materials provided by Helena, but not provided in the kit:**

- Tris-tricine Buffer (5 pkgs/box) 5358
- S.A.R.P. (10 x 1.0 mL) 5185
- Coagulation S.A.R.P. (10 x 1.0 mL) 5185
- Specially Assayed Control-1 (10 x 1.0 mL) 5301
- Sponge Wicks (2/pkg) 9015
- Titan Blotter Pads (100/pkg) 5037
- Microdispenser and Tubes (10 µL) 6210, 6211
- Development Water 5014
- Staining Dish 4061

**SUMMARY OF CONDITIONS**

- Lint-free tissues
- 0.85% Saline
- Magnetic stirrer
- Destaining solution: Thoroughly mix 350 mL water, 20 mL glacial acetic acid, 30 mL methanol,
- 30 mL glacial acetic acid.

**STEP-BY-STEP METHOD**

**A. Preparation of Standards and Test Plasmas**

1. Reconstitute one vial of S.A.R.P. with 1.0 mL deionized or distilled water. Make dilutions for preparation of the Standard Curve as follows:

<table>
<thead>
<tr>
<th>Percent</th>
<th>Parts</th>
<th>Parts</th>
<th>Activity Dilution</th>
<th>S.A.R.P.</th>
<th>0.85% Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>Use reconstituted S.A.R.P. undiluted</td>
<td>100%</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td>1:2</td>
<td>1</td>
<td>2</td>
<td>1</td>
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</table>

2. Dilute each patient sample and control with 0.85% saline. Prepare a 1:2 dilution (1 part patient sample and 1 part saline) and a 1:4 dilution (1 part patient plasma and 3 parts saline). Additional dilutions may be necessary depending on the patient history.Suspected abnormal samples may need to be tested undiluted.

**B. Preparation of Chamber**

1. Pour 65 mL Tris-tricine buffer into each inner section of the TITAN GEL chamber.

2. Plot the values of the standard curve versus each rocket height on the Rocket Antigen Report Form or on 3 cycle semi-logarithmic paper. Draw the line of best fit for the four points.

3. See Figures 1 and 2 for an example of a completed Rocket Plate and a standard curve drawn on a Rocket Antigen Report Form.

4. Destain the plate by placing it in Destaining Solution until the rockets can be distinguished easily. The background will still be a bluish purple color. If a clearer background is desired, the plate can be transferred to fresh destain after 30 minutes and left in the destain overnight.

5. Remove the plate from the Destaining Solution and rinse it briefly in deionized water.

6. Dry the plates at 37°C for 5 minutes or at room temperature until dry.

7. Place the plate on the lightbox or on a piece of white paper for easier viewing of the rockets. Mark the apex of each rocket peak with marker.

8. Using the Helena Rocket Ruler, measure the length of each peak in millimeters. The peak is measured from the top of each well to the apex of the rocket.

9. Plot the pattern of the rocket on the Rocket Antigen Report Form or on a 3 cycle semi-logarithmic paper.

10. Draw the line of best fit for the four points.

11. Dry the plates at 37°C for 5 minutes or at room temperature until dry.

**C. Application of Samples**

1. Place the sample to the refrigerator. Remove the plate lid and allow appropriately 5-20 minutes for the plate to equilibrate to room temperature and for excess buffer to be absorbed. Remove excess buffer from the wells, if necessary. Excess moisture in the plate can result in poor rockets.

2. Apply 10 µL of each dilution of the patient samples and controls to the designated wells, taking care not to damage the wells. Standard curve samples must be run on each plate.

3. Allow five (5) minutes for specimens to diffuse into the agarose.

4. Place the plate into the inner section of chamber, agarose side up, by gently squeezing the gel into place. Position the gel(s) so that the edges of the agar are in the buffer wells and the wells are toward the cathode (-) side of the chamber.

5. Cover the agarose with a single, lint-free tissue.

6. Place the plate on the lightbox or on a piece of white paper for easier viewing of the rockets. Mark the apex of each rocket peak with marker.

7. Place the plate on the lightbox or on a piece of white paper for easier viewing of the rockets. Mark the apex of each rocket peak with marker.

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10. Remove the plate from the Destaining Solution and rinse it briefly in deionized water.

11. Dry the plates at 37°C for 5 minutes or at room temperature until dry.

**D. Electrophoresis**

1. TITAN GEL Chamber

   Place the plate into the inner section of chamber, agarose side up, by gently squeezing the gel into place. Position the gel(s) so that the edges of the agar are in the buffer wells and the wells are toward the cathode (-) side of the chamber.

   Place the cover on the chamber. Electrophorese the plates at a constant current of 16 mA per plate for 3 hours.

   At the end of 3 hours, remove the plates from the chamber. Discard the buffer after each run.

2. Staining Procedure

   1. Rinse the plate with deionized or distilled water and wash it in 0.85% saline overnight with gentle stirring.

   2. After the overnight wash, rinse the plate with deionized or distilled water.

   3. Place the plate on a flat surface, agarose side up. Cover the agarose with a single, lint-free tissue.

   4. Place 2-3 Blotter Pads and a Development Weight on the plate for fifteen (15) minutes.

   5. Remove the Development Weight, blotters and tissue.

   6. Dry the plate in a laboratory drying oven at 70°C for 10-20 minutes. Do not over dry plates. The plate will be transparent when completely dry. If a dryer/oven is not available, the plates may be covered with wet lint-free tissues and allowed to dry at room temperature overnight or under a fan for 3 hours at room temperature as climate requires.

   7. When completely dry, scan the plate by immersing it in Rocket Stain for 5 minutes.

   8. Prepare the destaining solution by mixing together: 350 mL deionized water, 20 mL methanol, 30 mL glacial acetic acid.

   9. Destain the plate by placing it in Destaining Solution until the rockets can be distinguished easily. The background will still be a bluish purple color. If a clearer background is desired, the plate can be transferred to fresh destain after 30 minutes and left in the destain overnight.

   10. Remove the plate from the Destaining Solution and rinse it briefly in deionized water. Dry the plates at 37°C for 5 minutes or at room temperature until dry.

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**Measurements and Calculations**

1. Place the plate on the lightbox or on a piece of white paper for easier viewing of the rockets. Mark the apex of each rocket peak with marker.

2. Using the Helena Rocket Ruler, measure the length of each peak in millimeters. The peak is measured from the top of each well to the apex of the rocket.

3. Plot the pattern of the rocket on the Rocket Antigen Report Form or on 3 cycle semi-logarithmic paper. Draw the line of best fit for the four points.

4. See Figures 1 and 2 for an example of a completed Rocket Plate and a standard curve drawn on a Rocket Antigen Report Form.

**Figure 1:** Rocket patterns on a Protein C Antigen Rocket Plate. The lengths of the rockets (in millimeters) of the standard dilutions are used to prepare the standard curve. Patient results are read from the curve.

**Figure 2:** Representative standard curve prepared with S.A.R.P. on 3 cycle semi-logarithmic paper. Read the patient values from the standard curve and multiply each by the appropriate dilution factor. If S.A.R.P. is used to prepare the standard curve, the patient value must be multiplied by the assigned Protein C Antigen value of the appropriate lot of S.A.R.P. as well as the dilution factor.

**Example:**

- Patient value from curve = 30%
- Dilution factor = 2
- S.A.R.P. assigned value = 98%
- Actual Patient Factor

Protein C Antigen = 30% x 2 x 0.98 = 58.8%

6. Patient samples with Protein C Antigen levels greater than the range of the standard curve, must be reassayed using the appropriate dilutions.

**G. Quality Control**

A control material of known concentration, such as S.A.C. -1 (Cat. No. 5301) should be used on each run to verify all phases of the procedure and should be used on each gel. Refer to the package insert provided with the control for reconstitution instructions and assay values. If the control does not perform as expected, the patient results should be considered invalid or suspect.

**REFERENCE VALUES**

Protein C antigen values are usually expressed in relative percentage compared to a pooled normal plasma standard. The expected normal range for protein C antigen run by rocket electrophoresis has been reported at 65-129% with newborns showing less than 50% levels. Bertina et al., reported a range of 65-145% in healthy individuals with diminished levels found following anticoagulant therapy. There is apparently no difference in protein C antigen levels between healthy males and females.  

Helena tested 38 plasmas of presumed healthy men and women. The results were as follows:

<table>
<thead>
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<th>X</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>101%</td>
<td>24</td>
<td>53-149%</td>
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</table>

Each laboratory should establish its own normal range for this procedure.

**LIMITATIONS**

Most patients with congenital protein C deficiency show diminished levels of both immunologic and functional activity, the so-called type I deficiency. However, the finding of several patients with normal levels of protein C antigen and diminished functional protein C activity, type II deficiency; makes the diagnosis more difficult. The Helena procedure will detect only the type I deficiencies.

**TROUBLESHOOTING**

1. When applying the samples to the plate wells, do not allow the pipette tip to touch the sides of the wells.

2. Do not peel dry. Ovendrying will cause the plate to peel.
The Protein C Rocket EIA (electroimmunoassay) procedure is intended for the quantitative determination of plasma protein C antigen by Laurell rocket electrophoresis.  

**SUMMARY**

Protein C is a vitamin K dependent plasma protein that functions as a regulator of fibrin formation. In its activated form, it inhibits thrombin formation by the inactivation of active thrombin and factors V and VIII. A deficiency of protein C constitutes a thrombotic risk factor 1 of which superficial thrombophlebitis is the most common clinical feature.  

The virtual absence of plasma protein C has led to fatal thrombosis in neonates.  

**PRINCIPLES**

The Protein C Rocket EIA Procedure is performed in an agarose gel medium containing an antisera specific for Protein C. After the plasma samples are applied to the wells in the agarose, electrophoresis is used to migrate the proteins into the antibody field. A rocket-shaped precipitin pattern forms along the axis of migration. The length of this rocket pattern is proportional to the antigen concentration.

**REAGENTS**

- **Protein C Antigen Rocket Plates** (Cat. No. 5357)
- **Tris-tricine Buffer** (Cat. No. 5358)
- **Rocket Stain** (Cat. No. 5360)
- **Specialty Assayed Reference Plasma** (Cat. No. 5185)
- **Other Supplies and Equipment**

**Ingredient:**

- Protein C Antigen Rocket Plate (5 plates/box)
- Rocket Antigen Report Form (1 form/box)
- Sponge Wicks (2/pkg)
- TITAN GEL Chamber
- Microdispenser and Tubes (10 uL)
- TITAN GEL Sponge Wicks
- Staining Dish
- Development Weight

**For Sales, Technical and Order Information, and Service Assistance:**

Phone: 800-231-5663 toll free.

**Storage and Stability:**

- The material shows signs of dampness or discoloration.
- The material shows signs of discoloration.
- The expiration date on the package.

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

**Preparation for Use:**

- Dilute one package of buffer to 1000 mL with deionized or distilled water. The buffer is used for use when all material is completely dissolved.

**Storage and Stability:**

- The packaged buffer should be stored at room temperature (15 to 30°C) and is stable until the expiration date on the package.
- Diluted buffer is stable for two (2) months stored at 15 to 30°C.

**Signs of Deterioration:**

- Discard packaged buffer if the material shows signs of dampness or discoloration.
- Discard diluted buffer if it becomes turbid.

3. **Rocket Stain** (Cat. No. 5360)  
**Ingredients:**

- Rocket Stain is Coomassie Brilliant Blue.

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