The Helena Fibrinogen Assay Kit is intended for use in the quantitative determination of fibrinogen in human plasma.

**SUMMARY**
Fibrinogen, a high-molecular weight glycoprotein in plasma, plays a key role in hemostasis. Upon generation of thrombin, fibrinogen becomes the soluble polymer, fibrin. Sufficient fibrinogen must be present in the circulation to arrest bleeding and repair tissue should vascular trauma or injury occur. Thus, the quantitative determination of fibrinogen in plasma is important in the assessment of a thrombolytic disorder.

**RESULTS**
Twenty-five percent increasing in the plasma as a result of inflammation, pregnancy and oral contraceptive use. Decreased levels are found in certain pathological states including liver disease and disseminated intravascular coagulation (DIC). Congential deficiencies include afibrinogenemia (no detectable fibrinogen), hypofibrinogenemia (<1 mg/dL) and dysfibrinogenemia (abnormal fibrinogen).

**PRINCIPLES**
Laboratory has developed a simple method for the quantitative determination of fibrinogen by measuring the clotting time of dilute plasma following the addition of thrombin. At relatively high thrombin concentrations (>30 NIH units/mL) and low fibrinogen concentrations (2.0-3.0 mg/dL), the clotting time is dependent on the fibrinogen level. The thrombin clotting time, under these conditions, plotted on a log-log scale versus the fibrinogen concentration, is linear.

**REAGENTS**
1. **Helena Thrombin Reagent**
   - Ingredients: The reagent contains a lyophilized preparation of approximately 100 NIH units/mL of bovine thrombin with added stabilizers.

2. **Calcium Chloride**
   - Calcium Chloride solution contains 1.0% (w/v) calcium chloride, 3.2% (0.109 M).

**SPECIMEN AND COLLECTION**
- **Specimen**: Plasma obtained from whole blood collected with sodium citrate as an anticoagulant is the specimen of choice. The concentration of citrate should be 3.8% (0.129 M) or 3.2% (0.109 M).

- **Specimen Preparation**: Refer to the NCCLS guidelines H21-AZ on Specimen Collection and Preparation for Coagulation Studies. What should be collected into a plastic syringe, evacuated blood collection tube, a 2-syringe technique, or with a butterfly and syringe technique. Accurate coagulation studies depend on the correct whole blood to anticoagulant ratio. For blood samples with hemocrits (HCT) of >40-50%, normal, 9 parts of freshly collected citrated plasma should be added to one part anticoagulant. For blood samples with hemocrits outside the normal range, the amount of whole blood added to the anticoagulant according to the following formula:

   \[ \text{Parts whole blood to one part anticoagulant} = \frac{1}{1 - \text{HCT}} \times 9.0 \]

**PRODUCT INFORMATION**
- **Helena Fibrinogen Assay Kit**: Contains:
  - Helena Fibrinogen Assay Kit (Cat. No. 5376)
  - Helena Thrombin Reagent (Cat. No. 5370)
  - Helena Fibrinogen Calibrator (Cat. No. 5302)
  - Helena Anticoagulant (Cat. No. 5301)
  - Reaction cups (Cat. No. 5371)
  - Reaction vials (Cat. No. 5372)
  - Pipetman 2 (Cat. No. 5304)

**PROCEDURE**

**MATERIALS PROVIDED**
- **Laboratory**: Helena Fibrinogen Assay Kit
- **Helena Fibrinogen Assay Kit**: Cat. No. 5376
- **Helena Thrombin Reagent**: Cat. No. 5370
- **Helena Fibrinogen Calibrator**: Cat. No. 5302
- **Helena Anticoagulant**: Cat. No. 5301
- **Reaction cups**: Cat. No. 5371
- **Reaction vials**: Cat. No. 5372
- **Pipetman 2**: Cat. No. 5304

**SIGNIFICANCE OF DETERIORATION**
- Discard the buffer if visible signs of microbial contamination occur.

**INSTRUMENT**
- Any high quality electro-mechanical or photooptical coagulation instrument may be used such as the Helena Cascade® II, Cat. No. 1711.
The Helena Fibrinogen Assay Kit is intended for use in the quantitative determination of fibrinogen in human plasma.

**SUMMARY**

Fibrinogen, a high-molecular weight glycoprotein in plasma, plays a key role in hemostasis. Upon generation of thrombin, fibrinogen is converted to the insoluble polymer, fibrin. Sufficient fibrinogen must be present in the circulation to arrest bleeding and repair tissue should vascular injury or trauma occur. Thus, the fibrinogen concentration in plasma is important for accurate assessment of a thrombotic disorder.

**Fibrinogen Assay**

Fibrinogen values are limited to the plasma as a result of inflammation, pregnancy or oral contraceptive use. 

**Decreased levels** are found in conditions characterized by disease and disseminated intravascular coagulation (DIC). Congenital deficiencies include afibrinogenemia (no detectable fibrinogen), hypofibrinogenemia (=1 mg/dL) and dysfibrinogenemia (abnormal fibrinogen).

**PRINCIPLES**

Helena developed a simple method for the quantitative determination of fibrinogen by measuring the clotting time of dilute plasma following the addition of thrombin. At relatively high thrombin concentrations (>30 NIH units/mL) and low fibrinogen concentrations (20-30.0 mg/dL), the clotting time is dependent on the fibrinogen level. The thrombin clotting time, under these conditions, plotted on a log-log scale vs the fibrinogen concentration, is linear.

**REAGENTS**

1. **Helena Thrombin Reagent**

   **Ingredients**: The reagent contains a lyophilized preparation of 100 NIH units of bovine thrombin with added stabilizers.

   **CAUTION**: FOR IN-VITRO DIAGNOSTIC USE

2. **Reagent for Preparation for Use**

   **Preparation**: For release of a thrombin-calibrated assay, 2.0 mL or 0.5 mL of distilled or deionized water is added to the vial if it shows signs of precipitation or microbial contamination.

3. **Helena Fibrinogen Calibrator**

   **Ingredients**: The calibrator consists of a lyophilized citrated plasma, used as a reference material for fibrinogen assay by a functional clot assay. Refer to the enclosed package insert for the assay value.

   **CAUTION: FOR IN-VITRO DIAGNOSTIC USE**

The Helena Fibrinogen Calibrator has been found negative for Hepatitis B Antigen (HBsAg) and HIV antibody; however, this test does not show that there is no significant change in the fibrinogen levels on plasma samples stored up to 3 days at 2-8°C. Nevertheless, it is good laboratory practice to test samples as soon as possible after collection. Plasma may be stored at -20°C, or colder, for at most one month. Thermally stable at 37°C prior to testing.

**PROCEDURE**

**Materials Provided**

1. **Helena Fibrinogen Assay Kit**

2. **Helena Thrombin Reagent** (5 x 2.0 mL)

3. **Helena Fibrinogen Calibrator** (10 x 1.0 mL)

4. **Fibrinogen Graph Paper**

5. **Helena 1:10, 1:20, 1:30 and 1:40 dilutions of Fibrinogen Calibration**

6. **Owens' Veronal Buffer**

   **Ingested**: The buffer contains 28.4 mM barbital, 0.125 M sodium chloride and 0.05% sodium azide as a preservative.

   **CAUTION**: FOR IN-VITRO DIAGNOSTIC USE - DO NOT INGEST

7. **Preparation for Use**: The buffer is ready for use as packaged.

8. **Storage and Stability**: The buffer is stable until the date indicated on the label when stored at 2-8°C. Exercise care when piping to avoid contamination.

9. **Signs of Deterioration**: Discard the buffer if visible signs of microbial contamination occur.

**INSTRUMENT**

Any high quality electromechanical or photooptical coagulation instrument may be used such as the Helena Cascade® 4, Cat. No. 1171.

**SPECIMEN COLLECTION AND PREPARATION**

**Specimen**: Plasma obtained from whole blood collected with sodium citrate as an anticoagulant is the specimen of choice. The collection tube should contain 3.8% (0.129 M) or 3.2% (0.109 M) sodium citrate.

**Specimen Preparation**: Refer to the NCCLS guidelines H-21-AZ on Specimen Collection and Preparation for Coagulation Studies. Whole blood should be collected into a plastic syringe, evacuated blood collection tube, a 2-syringe technique, or with a butterfly and syringe technique. Accurate coagulation studies depend on this correct whole blood to anticoagulant ratio. For blood specimens with hematocrit (HCT) of 40-50%, normal, 9 parts of freshly collected plasma should be added to one part anticoagulant. For blood specimens with hematocrits outside the normal range, the amount of whole blood added to the anticoagulant according to the following formula:

$$\text{Parts whole blood to one part anticoagulant} = \frac{1}{1 - \text{HCT}}$$

**Particular care** should be taken when using evacuated blood collection tubes as they are designed to be used with 3 parts blood to 1 part anticoagulant. Therefore, if the hematocrit is abnormal, blood should be drawn into a syringe and an appropriate amount mixed with an adjusted volume of citrate anticoagulant. For greater stability of the specimen, an acid citrate anticoagulant solution should be prepared and utilized. The anticoagulant is prepared as follows:

1. **3 parts** of 0.1 M sodium citrate

2. **0.1 M citric acid

**Specimen Storage and Stability**: The allocoagulated whole blood specimen should be centrifuged at 1600-2000 x g for 10 minutes from the time of withdrawal. Plasma obtained by this method should be placed in a refrigerator. Plasma samples should be stored at 2-8°C in capped plastic test tubes. It should be noted that there is no significant change in the fibrinogen levels on plasma samples stored up to 3 days at 2-8°C. Nevertheless, it is good laboratory practice to test samples as soon as possible after collection. Plasma may be stored at -20°C, or colder, for at most one month. Thermally stable at 37°C prior to testing.

**EXPECTED VALUES**

The normal range of healthy adults contains about 150 to 350 mg/dL of fibrinogen. Helena tested thirty-two (32) presumed healthy donors and the following data was noted:

<table>
<thead>
<tr>
<th>Fibrinogen (mg/dL)</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9</td>
<td>101.4</td>
</tr>
<tr>
<td>2.9</td>
<td>101.4</td>
</tr>
<tr>
<td>3.9</td>
<td>101.4</td>
</tr>
</tbody>
</table>

**REFERENCES**

