K-ASSAY®

Fibrinogen + Calibrator

For the Quantitative Determination of Human Fibrinogen in Plasma
(Includes Fibrinogen calibrator for multi-point calibration)

Cat. No. KAI-035

INTENDED USE

For the quantitative determination of fibrinogen levels in disseminated intravascular coagulation (non-localized clotting within the blood vessels) and primary fibrinolysis (the dissolution of fibrinogen in a blood clot). FOR IN VITRO DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

Fibrinogen is a soluble precursor of the insoluble fibrin, the major component of a blood clot. It is long, 340,000 dalton glycoprotein composed of six subunits. When fibrinogen is activated by the hydrolytic enzyme thrombin, four subunits are removed. The remaining units polymerize into fibrin strands that form the basic structure of a blood clot. Most fibrinogen is intravascular, it is synthesized in the liver, approximately 2-5 grams per day.

Elevated levels of fibrinogen are associated with inflammation, trauma, surgery, and malignancy. Decreased levels are associated with congenital deficiencies or an increased use due to thrombosis or disseminated intravascular coagulation. The most common cause of low plasma fibrinogen is disseminated intravascular coagulation (DIC), a condition in which blood clots form throughout the microvascular system. DIC can be associated with some of the serious complications of childbirth. When fibrinogen levels fall to the point where blood is unable to clot, dangerous bleeding can occur. Fibrinogen levels below 100 mg/dL are associated with an increased risk of bleeding.

The K-ASSAY® Fibrinogen test is intended for the quantitative determination of human fibrinogen by immunoturbidimetric assay. The antigen used in the kit was produced against purified human fibrinogen. The fibrinogen antibody interacts with the fibrinogen in the plasma forming immune complexes. The immune complexes cause an increase in light scattering, which correlates with the concentration of plasma fibrinogen.

Fibrinogen has been measured using a variety of methods including radioimmunoassay (RIA), radial diffusion, nephelometric assay, and enzyme-linked immunosorbent assay. The K-ASSAY® Fibrinogen test uses an immunoturbidimetric assay format.

PRINCIPLE OF TEST

The K-ASSAY® Fibrinogen test quantifies the fibrinogen in the patient's plasma based on immunoturbidimetric assay. Calibration, controls, and patient samples are pipetted into sample cups. Microvolumes of samples and antibody diluent are automatically pipetted into individual cuvettes. Following an initial incubation and measurement of sample blank, antiserum is added to the cuvettes. The sample (antigen) solution and antiserum are then mixed in the reaction cuvettes. Insoluble antigen-antibody (immune) complexes form. The immune complexes cause an increase in light scattering, which correlates with the concentration of plasma fibrinogen. Following an incubation period lasting approximately 5 minutes, the absorbance of the solution is measured at 340 / 700 nm.

A calibration curve is generated by assaying a series of calibrators with known concentrations of proteins and using the instrument's data reduction capability to plot the change in absorbance versus concentration. Concentration of controls and patient samples are interpolated from the calibration curve. The antiserum used in the kit is a goat polyclonal antibody specific to human fibrinogen.

Calibrator A, B, C, and D should be prepared and used to make a calibration curve for quantifying the levels of fibrinogen present in the patient's plasma samples.

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Buffer Reagent

Trihydroxymethylaminomethane (100 mM) 2 x 20 mL

R2: Antisera Reagent

Anti-human fibrinogen goat antiserum (30%) 1 x 8 mL

Fibrinogen Calibrator (lyophilized) 1 x 1 mL

Approximately 340 mg/dL (1/21) human Fibrinogen

Exact value is indicated on calibrator vial label.

(*See section on International Standardization)

CAUTION: To avoid erroneous patient values, we recommend that fibrinogen measurements are performed uniformly on one type of plasma sample. Immediately after collection, centrifuge samples and remove plasma from cells.

Preparation of Reagents:

Discard reagents if they become contaminated. Evidence of cloudiness or particulate material in solution is cause to discard. Evidence of antibody when tested by FDA-accepted third generation methods. No known methods for HBsAG and HIV can offer total assurance that products derived from human blood will not transmit these diseases. Therefore, products derived from human blood and patient samples should be considered potentially hazardous and handled as if capable of transmitting infectious agents.

Fibrinogen + Calibrator

Potential biohazard material. Human source material. Treat as potentially infectious. All blood products are tested and found non-reactive for hepatitis B surface antigen (HBsAG) and HIV antibody when tested by FDA-accepted third generation methods. No known methods for HBsAG and HIV can offer total assurance that products derived from human blood will not transmit these diseases. Therefore, products derived from human blood and patient samples should be considered potentially hazardous and handled as if capable of transmitting infectious agents.

REAGENT PREPARATION

R1 and R2 are ready to use and do not require reconstitution.

Preparation of Calibrator Solution:

Add 1 mL of purified water to lyophilized calibrator to make calibrator stock solution. (Swirl gently to avoid foaming)

NOTE: Some analyzers automatically dilute the calibrators so the below manual dilution steps should not be performed. Check your instrument application sheet before proceeding with manual dilution.

Preparation of Calibrators:

1. Calibrator D: Dilute calibrator stock solution 3/21 in saline (ex. 300 µL calibrator stock solution + 1800 µL saline)

2. Calibrator C: Dilute a portion of Calibrator D 1/2 in saline (ex. 500 µL Calibrator D + 500 µL saline)

3. Calibrator B: Dilute a portion of Calibrator D 1/4 in saline (ex. 500 µL Calibrator D + 1500 µL saline)

Use Calibrator A, B, C, and D for calibration curve.

Calibration values input into the analyzer must take into consideration the 1/21 dilution of the sample in order to calculate the actual fibrinogen concentration in the original samples. The following formula should be used for this:

Calibration Value = Calibrator stock solution concentration x dilution ratio x 21 (for sample dilution)

Example: Calibrator D = 340 mg/dL x 3/21 x 21 = 1020 mg/dL

Calibrator Input Values should be input into the chemistry analyzer for Calibration A, B, C, D. FOR NIBSC standardized values, see section on International Standardization.

Calibrator Preparation Summary Table:

The following table shows how to make the 4 calibrators and calculate the input values using a calibrator stock solution concentration of 340 mg/dL. All users must calculate their own input values using the calibrator stock solution concentration for their lot of reagent.

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Cal D</th>
<th>Cal D Saline</th>
<th>Dilution Ratio</th>
<th>Calibration Input Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal A</td>
<td>1000 µL</td>
<td>7800 µL</td>
<td>3/21</td>
<td>0 mg/dL</td>
</tr>
<tr>
<td>Cal B</td>
<td>500 µL</td>
<td>500 µL</td>
<td>1/21</td>
<td>350 mg/dL</td>
</tr>
<tr>
<td>Cal C</td>
<td>500 µL</td>
<td>1500 µL</td>
<td>0.75/21</td>
<td>255 mg/dL</td>
</tr>
<tr>
<td>Cal D</td>
<td>300 µL</td>
<td>1800 µL</td>
<td>3/21</td>
<td>1020 mg/dL</td>
</tr>
</tbody>
</table>

STORAGE AND HANDLING

All reagents should be stored refrigerated (2-8°C). Return all reagents to 2-8°C promptly after use. Unopened reagents can be used for up to 18 months from the date of manufacture, as indicated by the expiration date on the package and bottle labels.

REAGENT STABILITY

Opened R1 and R2 can be used for 1 month if stored at 2-8°C. Discard reagents if they become contaminated. Evidence of cloudiness or particulate material in solution is cause to discard.

Reconstituted fibrinogen calibrator and the diluted calibrator solutions can be used for 1 week if stored at 2-8°C. However, calibrators should not be used if fibrin crystals are observed to have formed.

SPECIMEN COLLECTION AND PREPARATION

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Plasma is required for this assay.

Collect blood aseptically by venipuncture according to standard procedures in a tube containing sodium citrate. EDTA up to 500 mg/dL, or heparin can also be used.

NOTE: Unlike when using (liquid) sodium citrate tubes, there is no sample dilution with (dry) EDTA or heparin tubes. Therefore, fibrinogen values in EDTA plasma or heparin plasma will be higher.

CAUTION: To avoid erroneous patient values, we recommend that fibrinogen measurements are performed uniformly on one type of plasma sample.

Immediately after collection, centrifuge samples and remove plasma from cells.

Dilute plasma 1/21 with saline. (Ex. 50 µL plasma + 1000 µL saline)

Generally, separated plasma should remain at room temperature for no longer than 8 hours. After 8 hours, the plasma should be refrigerated at 2-8°C. If the sample is not assayed within 48 hours, it should be frozen at -20°C.


Use plastic tubes for storing the sample, do not use glass.

K-ASSAY® Fibrinogen + Calibrator

1 Rev. 2017-02-01

K-ASSAY® Fibrinogen + Calibrator

2 Rev. 2017-02-01
Fibrinogen


**PROCEDURE**

Materials Supplied

- Reagent 1 (R-1) Buffer Reagent 2 x 20 mL
- Reagent 2 (R-2) Antiserum Reagent 1 x 8 mL
- Fibrinogen Calibrator (lyophilized) 1 x 1 mL

Materials Required But Not Supplied

- Two-reagent clinical chemistry analyzer capable of accurately dispensing the required volumes, reading at 340 and 700 nm, and maintaining 37°C.
- For reconstitution of calibrator:
  - Saline
  - Purified water
  - Pipette capable of dispensing the required volumes
  - Test tubes or appropriate vials for storage of diluted calibrator

**Assay Procedure**

An example of an automated application (Hitachi 717):

<table>
<thead>
<tr>
<th>Sample</th>
<th>6 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>↓</td>
</tr>
<tr>
<td>+ → R1</td>
<td>250 µL</td>
</tr>
<tr>
<td>↓ 37°C</td>
<td>5 min.</td>
</tr>
<tr>
<td>+ → R2</td>
<td>50 µL</td>
</tr>
<tr>
<td>↓ 37°C</td>
<td>5 min.</td>
</tr>
</tbody>
</table>

Note: Allow all reagents and specimens to warm to room temperature. Mix all reagents gently before using.

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

**INSTRUMENT** Hitachi 717
**TEMPERATURE** 37°C
**TEST** FIB
**ASSAY CODE** (2 POINT) - (24) - (50)
**SAMPLE VOLUME** (6) - (1)
**R1 VOLUME** (250) - (1) (NO)
**R2 VOLUME** (50) - (1) (NO)
**WAVELENGTH** (703) - (340)
**CALIB. METHOD** (NONLINEAR) (1) (4)
**STD.(1) Conc.-POS.** ("1") - (1)
**STD.(2) Conc.-POS.** ("2") - (2)
**STD.(3) Conc.-POS.** ("3") - (3)
**STD.(4) Conc.-POS.** ("4") - (4)
**STD.(5) Conc.-POS.** (0) - (0)
**STD.(6) Conc.-POS.** (0) - (0)
**SD LIMIT** (999)
**DUPLICATE LIMIT** (100)
**SENSITIVITY LIMIT** (0)
**ABS. LIMIT (SLOPE)** (32000) (INCREASE)
**PROZONE LIMIT** (32000) (LOWER)
**EXPECTED VALUE** (-99999) (99999)
**PANIC VALUE** (-99999) (99999)
**INSTRUMENT FACTOR** (1.00)

1-4 Input concentration of calibrators
Parameters for other automated analyzers are available.

**CALIBRATION**

It is recommended that a multi-point calibration curve be made using the calibrator provided. It is recommended that the user determine calibration frequency, as this will depend on the instrument and type/number of assays being run. Initially, calibration should be performed each day.

**INTERNATIONAL STANDARDIZATION**

If the user wishes to calculate or report results consistent with the National Institute for Biological Standards and Control (NIBSC) international standard for plasma fibrinogen (89/644) the calibrator values for calibrators A, B, C, and D should be multiplied by 0.81 before entering these values into the analyzer.

Alternatively, if the calibrator values have not been changed, final fibrinogen assay results can be multiplied by 0.81.

Example:

If the calibrator values have not been changed, a fibrinogen result of 300 mg/dL would be reported as 243 mg/dL standardized to NIBSC plasma fibrinogen standard.

(300 mg/dL x 0.81 = 243 mg/dL)

**QUALITY CONTROL**

Normal and abnormal controls of known concentration should be included with every assay performed. The value determined for the controls should fall within the stated limits of the values assigned to the controls. The validity of the assay is in question if the values for the controls generated by the assay’s calibration curve does not fall within this range. Recalibrate if the values determined for the controls fall outside the stated range.

**LIMITATIONS OF PROCEDURE**

The measurable range for fibrinogen is 100 to 900 mg/dL. Grossly lipemic samples and samples with very high triglyceride concentrations should be diluted an additional 1/2 with isotonic saline or filtered to decrease nonspecific light scattering. If fibrinogen concentration is above highest calibrator value, dilute 1 part sample with 4 parts isotonic saline and re-assay. Multiply results by 5 to compensate for dilution.

Samples from patients under hyperfibrinolysis need to be tested promptly as they may decompose and thus have a shorter sample shelf life. Studies have not been done to determine the effect of high levels of fibrinogen degradation products (FDP) on the measurement of fibrinogen using this test kit. Very high levels of FDP may cause interference.

**PERFORMANCE**

Sensitivity

When a saline blank is used as a sample, the absorbance is below 0.050. When a calibrator having a fibrinogen concentration of around 248 mg/dL is assayed, the absorbance (after subtracting the saline blank) is within 0.050 to 0.150.

Specificity

When control serum with a known value is assayed, the result is within ±10% of the assigned value.

**REFERENCES**


**LABELING SYMBOLS**

**EU AUTHORIZED REPRESENTATIVE**

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**ORDERING / PRICING / TECHNICAL INFORMATION**

Rev. 2017-02-01

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