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 a 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.[1]

Elevated levels of fibrinogen are associated with inflammation, trauma, surgery, and malignancy.[2] Decreased levels are associated with congenital deficiencies or an increased use due to thrombosis or disseminated intravenous 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 antiserum 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.[2,3] 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. Calibrators, 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 Tris(hydroxymethyl)aminomethane (100 mM)	2 x 20 mL
R2: Antiserum Reagent Anti-human fibrinogen goat antiserum (30%)	1 x 8 mL
Fibrinogen Calibrator (lyophilized) Approximately 340 mg/dL* (1/21) human Fibrinogen	1 x 1 mL

Exact value is indicated on calibrator vial label.
(*See section on International Standardization)

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE. Rx only.

Not to be used internally in humans or animals. Normal precautions exercised in handling laboratory reagents should be followed. Do not pipette by mouth. Avoid ingestion and contact with skin.

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

To avoid erroneous patient values, we recommend that fibrinogen measurements are performed uniformly on one type of plasma sample (see "SPECIMENT COLLECTION AND PREPARATION" for more information).

Do not mix or use reagents from one test kit with those from a different lot number.

Do not use reagents past their expiration date stated on each reagent container label.

Reagents in this kit contain sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control, Atlanta, GA, 1976.

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 Stock 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)
4. Calibrator A: Use saline only.

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 Input 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

Calibration Input Values should be input into the chemistry analyzer for Calibrator 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.**

	Calibrator Stock	Cal D	Saline	Dilution Ratio	Calibration Input Value
Cal. D	300 µL		1800 µL	3/21	1020 mg/dL
Cal. C		500 µL	500 µL	1.5/21	510 mg/dL
Cal. B		500 µL	1500 µL	0.75/21	255 mg/dL
Cal. A			1000 µL	0	0 mg/dL

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.

Samples should not be repeatedly frozen and thawed. (NCCLS document H18-A, vol. 10, no. 12, p. 12, 1990).

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

AUTOMATED ANALYZER APPLICATION

Suitable for two-reagent automated analyzers that use a multi-point calibration method.

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 automated application (Hitachi 717):

Sample	6 µL
↓	
• ← R1 (Buffer Reagent)	250 µL
↓ 37°C, 5 min.	
• ← R2 (Antiserum Reagent)	50 µL
↓ 37°C, 5 min.	

2-point endpoint, 340/700 nm

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) ( )
R1 VOLUME	(250) ( ) (NO)
R2 VOLUME	(50) ( ) (NO)
WAVELENGTH	(700) (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	(10000)
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 fibrinogen 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 reassay. 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.

Precision

When a sample containing 200 mg/dL fibrinogen is assayed 20 times (within-run), the absorbance C.V. is below 5%.

Precision Assay: Within Run

	Sample A	Sample B	Sample C
N	20	20	20
MAX	152	456	763
MIN	136	429	721
AVE	142.4	444.7	739.6
CV%	2.96	1.48	1.43

Precision Assay: Between Runs

	Sample A	Sample B	Sample C
N	10	10	10
MAX	170	329	571
MIN	165	316	544
AVE	167.4	323.6	554.1
CV%	0.94	1.06	1.50

Accuracy / Correlation

A comparison of the **K-*ASSAY*®** Fibrinogen test and an Incstar Fibrinogen test kit were performed on a Hitachi 717. The test results provided the following data:

y = 0.967x + 33.91
r = 0.995
n = 50
x = Incstar ITA
y = **K-*ASSAY*®** Fibrinogen

Additional correlation studies on assays using the Clauss method (clotting method) are available.

Assay Range

100-900 mg/dL or
81-729 mg/dL (using NIBSC international standard)

INTERFERENCE

Bilirubin C:	No interference up to 25 mg/dL
Hemoglobin:	No interference up to 500 mg/dL

EXPECTED VALUES

In our laboratory, the expected values of fibrinogen in citrated plasma and EDTA plasma was determined. Blood was collected in tubes containing (liquid) sodium citrate and in tubes containing (dry) EDTA from 80 healthy individuals.

The correlation coefficient between the two collection methods was as follows: r = 0.980.

The expected values obtained were:

Citrated Plasma:	196 – 441 mg/dL
EDTA Plasma:	244 – 539 mg/dL

If using NIBSC international standardization, these ranges should be appropriately adjusted as explained in the "International Standardization" section

Due to patient population differences as well as variations in analyzers and other factors, it is recommended that each laboratory determines its own expected range.

REFERENCES

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LABELING SYMBOLS

LOT	Lot Number
RGT	Reagent
STD	Calibrator
	Expiration or "Use By" Date
REF	Catalog Number
IVD	For *In Vitro* Diagnostic Use
2-8°C	Temperature Limitation. Store between 2 and 8 degrees C
	Potential Human Biohazard
	Manufacturer
	Consult Package Insert for Instructions for Use
EC REP	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

CE

EC REP

Advena Ltd.
Tower Business Centre, 2nd Flr.,
Tower Street, Swatar, BKR 4013 Malta

ORDERING / PRICING / TECHNICAL INFORMATION

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive
Seattle, WA 98168 USA
TEL: (206) 575-8068 / (800) 526-4925
FAX: (206) 575-8094