FIBRIPHEN™

REF CK571K R 6 x 1 mL
REF CK572K R 6 x 2 mL
REF CK575K R 8 x 5 mL

IVD

Clotting method for quantitative determination of Fibrinogen.

HYPHEN BioMed
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INTENDED USE:

The FIBRIPHEN™ kit is a clotting method for *in vitro* quantitative determination of Fibrinogen in human citrated plasma (Clauss method), using manual or automated method.

SUMMARY AND EXPLANATION:

Technical:[1,2]
Fibrinogen is a 340 Kd soluble plasma glycoprotein, synthesized in the liver, containing 6 peptidic chains, with a 2 to 2 symmetry, and linked by disulfide bridges (2 Aα, 2 Bβ and 2 γ chains). Thrombin clots fibrinogen and forms fibrin, which is then stabilized by activated Factor XIII in presence of calcium. Fibrinogen is lysed by plasmin to fragments X and Y, first, then D and E.
Clinical:[2-6]
Fibrinogen concentration in normal human plasma is usually in the range 2 to 4 g/L. Elevated fibrinogen concentrations (> 4g/L) are observed in clinical situations associated with inflammation and have also been considered as a risk factor for cardiovascular disease and thrombosis.
Hypofibrinogenemia is mainly associated with severe liver disease, or excessive consumption of fibrinogen (DIC, hyperfibrinolysis).
Numerous variants of fibrinogen have been described, associated to asymptomatic cases, or to cases with bleeding and/or thrombosis.

PRINCIPLE:

In the presence of a constant and in excess amount of thrombin, the clotting time (CT) obtained for diluted citrated plasma depends on the plasma fibrinogen concentration.

REAGENTS:

R **Calcium Thrombin**, from bovine origin (about 100 NIH/mL), lyophilized. Contains BSA, an heparin neutralizing substance and stabilizers.

FIBRIPHEN™ 1
REF **CK571K ➔ 6 vials of 1 mL.**
FIBRIPHEN™ 2
REF **CK572K ➔ 6 vials of 2 mL.**
FIBRIPHEN™ 5
REF **CK575K ➔ 8 vials of 5 mL.**

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of animal origin. Users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

REAGENT PREPARATION:

Gently remove the freeze-drying stopper, to avoid any product loss when opening the vial.

R Reconstitute the contents of each vial with exactly:
REF **CK571K ➔ 1 mL of distilled water.**
REF **CK572K ➔ 2 mL of distilled water.**
REF **CK575K ➔ 5 mL of distilled water.**

Shake vigorously until complete dissolution while avoiding formation of foam and load it directly on the analyzer following application guide instruction.
For manual method, allow to stabilize for 30 minutes at room temperature (18-25°C), homogenize before use.

STORAGE AND STABILITY:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

R Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:

- **14 days** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **1 month** frozen at -20°C or less*
- **Stability on board of the analyzer: see the specific application.**

*Thaw only once, as rapidly as possible at 37°C and use immediately.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- Imidazole Buffer (AR021B/AR021K/AR021L/AR021M/AR021N).
- Specific calibrators and controls with known titration, such as:

Product Name	Reference
BIOPHEN™ Plasma Calibrator	222101
BIOPHEN™ Normal Control Plasma	223201
BIOPHEN™ Abnormal Control Plasma	223301
EASYPLASMA™ Control Set	225601
EASYPLASMA™ Calibrator	226601

Also refer to the specific application guide of the analyzer used.

Materials:

- Electromagnetic water-bath, semi-automatic or automatic analyzer for clotting assays.
- Stopwatch; Calibrated pipettes; plastic test tubes.

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. Discard the first tube.
Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[7] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references.[7,8]

PROCEDURE:

The kit can be used in manual or automated method. Perform the test at **37°C** and the clotting time, triggered by addition of FIBRIPHEN™ reagent, is measured.

For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.

Assay method:
1. Reconstitute the calibrators and controls as indicated in the specific instructions. Prepare 2 mL of calibrator **diluted 1:5** in Imidazole buffer (note: by definition, the 1:20 dilution of the calibrator corresponds to a concentration of "C" g/L of Fibrinogen). For the calibration curve, dilute the calibrator in Imidazole buffer as described below ("C" defines the concentration of Fibrinogen):

Fibrinogen (g/L)	C:2	C	2C	4C
Dilution	1:40	1:20	1:10	1:5
Volume of calibrator at 1:5	0.125mL	0.250mL	0.500mL	1mL
Volume of Imidazole Buffer	0.875mL	0.750mL	0.500mL	0mL

2. Dilute the specimens, calibrators and controls in Imidazole buffer, as described in the table below:

Specimens	References	Dilution
Controls	223201 / 223301 / 225601	**1:20**
Specimens to test	NA	**1:20**

Establish the calibration curve and test it with the quality controls. If stored at room temperature (18-25°C), test the diluted specimens quickly. The exact calibrator and control concentrations for each batch are indicated on the flyer provided with the kit.

To ensure optimal performances of the assay, perform all assays (calibration, samples, controls) extemporaneously and successively without interruption.

3. Introduce into a reaction cuvette, plastic test tube incubated at **37°C:**

	Volume
Calibrator, specimens or controls diluted	200 µL
Incubate **at 37°C** for **2 minutes**, then add the (starting the stop-watch):	
R **Calcium Thrombin** pre-incubated at **37°C**	100 µL
Record the exact clotting time CT (in seconds).	

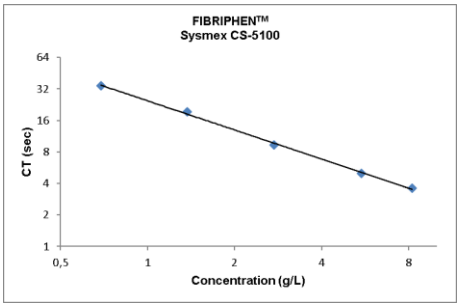
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

The FIBRIPHEN™ assay can be calibrated for the assay of fibrinogen. The calibrator covering the calibration range is available from HYPHEN BioMed (see the REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED paragraph) and can be used to establish the calibration curve.

- The calibration range is about 0.7 to 7.5 g/L (on CS-series).

The calibration curve shown below is given by way of example only. The calibration curve established for the assay series must be used.



QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.
Include the quality controls with each series, as per good laboratory practice, in order to validate the test. A new calibration curve should be established, preferably for each test series, and at least for each new reagent batch, or after analyzer maintenance, or when the measured quality control values fall outside the acceptance range for the method.
Each laboratory must define its acceptance ranges and verify the expected performance in its analytical system.

RESULTS:

- For the manual method, plot the calibration curve log-log, with the clotting time (sec) along the Y-axis and the Fibrinogen concentration, expressed as g/L, along the X-axis.
- The concentration of Fibrinogen (g/L) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- If other dilutions are used, the level obtained should be multiplied by the additional dilution factor used.
- The results should be interpreted according to the patient's clinical and biological condition.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- Various drugs or treatments can affect the results. An additional investigation should be realized to determine the origin of each unexpected or abnormal result. Diagnosis of dysfibrinogenemia must always be combined with a fibrinogen antigenic assay. Recovery of therapeutic fibrinogen concentrates can also be impacted by the type of reagent used, and appear weaker with bovine thrombin (eg FIBRIPHEN™) compared to human thrombin[9].
- The obtained CT for a same sample and a same reagent lot can vary according to the instrument used and the clot detection mode.
- If obtained CT is too short (high concentration of fibrinogen), dilute more the plasma. If obtained CT is too long (low concentration of fibrinogen), dilute less the plasma.

EXPECTED VALUES:

The normal plasma Fibrinogen level in the adult population is usually in the range of 2 to 4 g/L[5]. However, each laboratory has to determine its own normal range.

PERFORMANCES:

- The measuring range depends on the analytical system used (about 0.4 to 13 g/L of fibrinogen on CS-series, with redilution).
- For a better accuracy, samples measured ≤ 1 g/L can be tested at the twice-concentrated dilution, and obtained results divided by 2; for elevated samples (above calibration), an additional two-fold dilution can be used and obtained results multiplied by 2.
- Performance studies were conducted internally on Sysmex CS-5100. Performance was assessed using laboratory controls over a 5-day period, 2 series per day and 3 repetitions within each series for a control level. The following results were obtained:

Control	**Intra assay**				**Inter assays**			
	n	**Mean**	**CV%**	**SD**	**n**	**Mean**	**CV%**	**SD**
Control 1	40	3.03	1.4	0.04	30	3.04	0.9	0.03
Control 2	40	1.60	1.6	0.03	30	1.55	2.6	0.04

- Correlation with reference method (Dade® Thrombin Reagent vs FIBRIPHEN™ on Sysmex CS-5100) :
  n = 150 y = 1.06x – 0.04 r = 0.999
- Interferences:
  No interference, on the analyzer Sysmex CS-5100 was observed with the molecules and up to following concentrations:

Hemoglobin	**Bilirubin (F/C)**	**Heparins (UFH/LMWH)**
1000 mg/dL	60 mg/dL	2 IU/mL
Rivaroxaban / Apixaban / Edoxaban / Dabigatran		
400 ng/mL		
FDP	**Intralipids**	**Argatroban**
130 µg/mL	1000 mg/dL	400 ng/mL

Also refer to the specific application guide of the analyzer used.

REFERENCES:

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SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

Changes compared to the previous version.