

FIBRIPHEN™ LRT
REF CK585K-RUO

R 6 vials x 5 mL

FOR RESEARCH USE ONLY.
DO NOT USE IN DIAGNOSTIC PROCEDURES.

English, revision: 07-2024

INTENDED USE:

Clotting method based on Clauss method for the *in vitro* quantitative determination of Fibrinogen in human citrated plasma, using a manual or automated method.
This kit is for research use only and must not be used for patient diagnosis or treatment.

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 A α , 2 B β 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.

PRINCIPLE:

In the presence of a constant and in excess amount of bovine thrombin, the clotting time (CT) obtained for diluted citrated plasma is inversely proportional to the plasma fibrinogen concentration.

REAGENTS:

R **Calcium Thrombin** (bovine origin), at approximately 80 NIH/mL, liquid form. Contains BSA, a heparin neutralizing substance, preservatives and stabilizers.

The product is classified as non-hazardous and is not subject to labeling according to EC Regulation No. 1272/2008 [CLP].

WARNINGS AND PRECAUTIONS:

- This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.
- Waste should be disposed of in accordance with applicable local regulations.
- This device of *in vitro* use is intended for professional use in the laboratory.

REAGENT PREPARATION:

R Reagent is ready to use; homogenize 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 opening, free from any contamination or evaporation, and stored closed, is of:

- 90 days** at 2-8°C.
- 7 days** at room temperature (18-25°C).
- Stability on board of the analyzer: see the specific Application Guide.**

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:
Reagents:

- Imidazole buffer (AR021B-RUO/AR021K-RUO/AR021L-RUO/AR021M-RUO/AR021N-RUO) or Hemostasis Hepes Buffer (AR033K-RUO/AR033L-RUO/AR033M-RUO/AR033N-RUO). Use the same buffer for all dilutions performed.
- Specific calibrators and controls:

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

- Automatic analyzer for chromogenic assays such as: CS-series, STA-R® family, ACL-TOP® family, CN-series.
- Laboratory material.

Materials (endpoint method):

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

Please note that the applications on other analyzers can be validated by the instrument manufacturer under their responsibility.

TRACEABILITY:

Certificates of traceability and Instructions for Use of above calibrators and controls are available on the HYPHEN BioMed website. For more information refer to Instructions for Use of above calibrators and controls.

SPECIMEN COLLECTION AND PREPARATION:

Collection, preparation and storage of Platelet Poor Plasma (PPP) should be made according to laboratory or other validated methods²⁻⁵.

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. According to CLSI H21-A5⁵ and studies⁵:

- Plasma should remain at room temperature for no longer than 4 hours.
- If assays will not be completed within 4 hours, plasma should be frozen at -20 °C or below.
- Plasma samples should be thawed at 37°C, only once.

PROCEDURE:

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

Automated method:

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 calibrator and control as indicated in the specific instructions. Calibrator should be diluted in the Imidazole buffer or Hemostasis Hepes Buffer. Prepare calibration points on the range about 0.7 to 7 g/L (C:4 – C:2 – C – 2C-8C/3 fibrinogen in Imidazole buffer or Hemostasis Hepes Buffer) for working dilution 1:10 (alternatively C:2 – C – 2C – 4C for working dilution 1:20 with manual method).

The 1:10 dilution (in automated method) or 1:20 (in manual method) of calibrator correspond to the "C" g/L concentration of fibrinogen ("C" defines the concentration of fibrinogen for commercial calibrator).

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

Specimens	References	Dilution	
		Manual method	Automated method
Controls	223201-RUO / 223301-RUO / 225601-RUO	1:20	1:10
Specimens to test	NA	1:20	1:10

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 at 37°C:

	Volume
Calibrator, specimens or controls diluted	100 μ L
Incubate at 37°C for 3 minutes , then add the following (starting the stop-watch):	
R Calcium Thrombin pre-incubated at 37°C	50 μ L
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.

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 endpoint 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 (reported in 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 obtained should be for research use only and must not be used for patient diagnosis or treatment.

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 no limp appearance or showing signs of contamination must be rejected.
- Anti-thrombin substances may interfere in Fibrinogen Clauss assays³.
- User defined modifications are not supported by HYPHEN BioMed as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in HYPHEN BioMed Application Guides or these Instructions for Use.

PERFORMANCES:

Performances studies were conducted on analyzers. The following performance data represent typical results and are not to be regarded as specifications for FIBRIPHEN™ LRT. Mathematical analyses are performed using a validated statistical software built in accordance with state of the art.

Analytical performances

Measuring Range

The measuring range is defined by the analyzer system used and is documented in the respective Application Guides of the analyzers.

Accuracy

Accuracy studies were assessed using laboratory controls and pooled plasmas. Trueness: bias is less than 9% for all samples. Precision: coefficient of variation (CV) for all samples is less than 6% or repeatability, less than 9% for reproducibility and less than 9% for within laboratory. Precision is documented in the respective Application Guides of the instruments.

Interfering substances

Interferences are defined by the analyzer system used and are documented in the respective Application Guides of the analyzers.

REFERENCES:

1. Mosesson M.W. Fibrinogen and fibrin structure and function. JTH. 2005
2. Marguerie G. Le fibrinogène, facteur multifonctionnel de l'hémostase. Médecine/Sciences. 1986.
3. CLSI Document H30-A2: "Procedure for the determination of Fibrinogen in plasma; approved guideline-Second edition". 2001.
4. CLSI Document CLSI H21-A5: "Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays; approved guideline". 2008
5. Ieko M. et al. Expert consensus regarding standardization of sample preparation for clotting time assays. Int J Hematol. 2020.

For customer support or Application Guides, please contact your local provider or distributor (see www.hyphen-biomed.com).

Changes compared to the previous version.

The following symbols may appear on the product labeling:

REF	Catalogue number	LOT	Batch code	RUO	Product for <i>in-vitro</i> research use, only
Rx	Numerical < x> identification of reagent		See instructions for use	WHO STD	WHO standard code
	Temperature limitation		Manufacturer		YYYY-MM-DD Use by
	Biological risks		Reconstitution volume	CONTENTS	Contents
Cx	Numerical < x> identification of control	i-MA	See instructions in Method Application guide	CONTAINS	Contains
EXP	Expiration date		Contains sufficient for <n> tests	UNIT	Measurement unit
TARGET VALUE	Target Value		Keep away from sunlight and heat	CALx	Numerical < x> identification of calibrator
	Contains biological material of animal origin		Contains human blood or plasma derivatives	DANGER	Danger
WARNING	Warning	CONTROL+	Positive control	CONTROL-	Negative control
ACCEPTANCE RANGE	Acceptance range				