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Fibriphen 1

 # CK571K-RUO

Thrombin reagent for the quantitative clotting assay of Fibrinogen

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

Last revision : 25/06/2014

1. Intended use:

The **Fibriphen 1** kit is a thrombin reagent proposed for the quantitative determination of Fibrinogen in human citrated plasma using a clotting method (Clauss method). **This kit is for research use only and should not be used for patient diagnosis or treatment.**

2. Assay principle:

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

3. Assay specimen:

Human plasma obtained from Trisodium Citrate anticoagulated blood.

4. Reagents:

Each kit contains **6 vials of 1 ml** of reagent, containing calcium thrombin from bovine origin (about 100 NIH/ml), lyophilized in presence of an heparin neutralizing substance, preservatives and stabilizers.

Note: Source bovine plasma used for the preparation of **the reagent** and BSA were tested with registered methods and found negative for infectious diseases, especially for BSE. However, no assay may warrant the total absence of infectious agents. Any product of biological origin must then be handled with all the required cautions, as being potentially infectious.

5. Reagents and material required, but not supplied:

- Pipettes with dispensing volumes from 20 µl to 1,000 µl.
- Semi-automatic or automatic coagulation instrument, or fibrometer or electromagnetic water bath.
- Distilled water.
- Imidazole buffer (# AR021A/AR021K/AR021L).
- Reference normal citrated human plasma pool, or plasma calibrator titrated for Fibrinogen (BIOPHEN Plasma Calibrator - # 222101).
- Normal and Abnormal quality control plasmas, titrated for Fibrinogen (BIOPHEN Normal Control Plasma - #223201 and BIOPHEN Abnormal Control Plasma – #. 223301).

6. Reagent preparation and stability:

In the original package, and before any use, when stored at 2-8°C, the reagent is stable until the expiration date printed on the kit.

- **Reagent Preparation**:

Restore each vial with **1 ml** of distilled water; mix gently until complete dissolution of the content (vortex), let to stabilize for about 30 min. at room temperature (18-25°C); homogenize before each use.

- **Reagent stability following reconstitution:**

– 7 days at room temperature (18-25°C).
– 14 days at 2-8°C.
– 1 month frozen at -20°C or below

In the original vial, provided that any contamination or evaporation is avoided.

7. Sample collection and preparation:

Blood (9 vol.) must be collected on 0.109M (or 0.129M) trisodium citrate anticoagulant (1 vol.); plasma supernatant is decanted following a 15 min. centrifugation at 2,500 g. Citrated plasma must be tested within 4 hours when stored at room temperature (18-25°C), or it can be frozen at –20°C or below for up to 1 month. Just before use, the plasma must be thawed for 15 min. in a water bath at 37°C.

Refer to GEHT or NCCLS guidelines for further instructions on specimen collection, handling and storage. Discard any plasma presenting an unusual aspect (haemolysed, lipaemic aspect….).

8. Protocol:

- **Calibration curve:**

The calibration curve can be established with a normal citrated human plasma pool with a determined Fibrinogen concentration ("C" g/L), or with BIOPHEN Plasma Calibrator (# 222101), using the Fibrinogen concentration (C) indicated on the flyer for the lot used.
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). Using this 1:5 preparation, the calibration curve is obtained as follows:

Fibrinogen (g/L)	C:2	C	2C	4C
Dilution	**1:40**	**1:20**	**1:10**	**1:5**
Calibrator dil. 1:5	0.125 mL	0.250 mL	0.500 mL	1 mL
Imidazole Buffer	0.875 mL	0.750 mL	0.500 mL	0mL

The calibration curve must be used within 2 hours at room temperature (18-25°C).

- **Preparation of tested plasma, and quality controls:**

Tested plasma must be **diluted 1:20** in Imidazole buffer. The diluted plasma must be tested within 2 hours.

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

- **Assay:**

Mechanical manual method:

Principle: a mechanical coagulation indicator, such as a metal ball or index, or balancing, is used for detecting clotting. The test is performed at 37°C.

Preincubate the reagent at 37°C.

Into a small test tube, or in the reaction cuvette of the coagulation instrument, introduce:

- **200 µL** of calibration solution, or of tested plasma diluted **1:20**.
- Incubate for **2 min. at 37°C**, and then introduce (starting the stop-watch):
- **100 µl of Fibriphen reagent** (preincubated at 37°C).

Record the clotting time CT (in seconds).

Automatic Method:

The assay can be used with the semi-automatic or automatic instruments, such as STA-R, KC-10, etc…

The usual program used for testing Fibrinogen by clotting assay (Clauss method) can be applied. The respective specimen and reagent volume ratios indicated for the manual method must be strictly respected. Usually, with automatic methods the volumes used for reagents and diluted tested plasma are half those recommended for the manual method.

With semi automatic or automatic instruments, especially those with a photometric detection of clot formation, obtained clotting times can be slightly different from those obtained with the manual method.

Adaptations on the main coagulation analyzers are available upon request.

9. Expression of results:

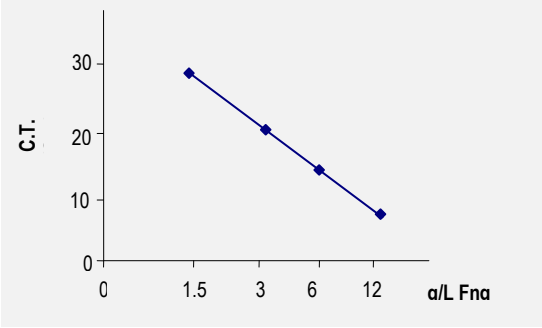
On a bi-logarithmic graph paper, plot on abscissae the Fibrinogen concentrations (in g/L) and on ordinates the corresponding clotting times (in sec).

The Fibrinogen concentration in the tested sample (diluted 1:20) is directly obtained on the calibration curve. Results are expressed in g/L Fibrinogen.

Using automated methods, the Fibrinogen concentrations are directly calculated by the analyser, respectively to the calibration curve, and the sample dilution used.

10. Example of Calibration curve:

This calibration curve, obtained using the manual method, is indicated as an example only.



Only the calibration curve generated for the series of assays performed must be used for calculating the results.

11. Quality Control:

Using quality control plasmas, titrated for Fibrinogen, allows validating the calibration curve, as well as the homogeneous reactivity from run to run, when using a same lot of reagents. Various control plasmas are available:

BIOPHEN Normal Control Plasma: (ref 223201).

BIOPHEN Abnormal Control Plasma : (ref. 223301).

The clotting time obtained for a repeat test and with the same reagent lot can slightly vary according to the instrument used and the clot detection mode and sensitivity adjustment. Each laboratory should establish and validate its own usual range, as well as acceptance ranges, in its specific test conditions.

12. Interferences and limits:

Various drugs or therapies can affect the results (eg: anti-thrombin substances may interfere in the assay and prolong the obtained clotting time). An additional investigation should be conducted to determine the origin of each unexpected or abnormal result.

The results obtained should be for research purposes only and not used for patient diagnosis or treatment.

A "repeat" clotting time for a sample even with the same reagent lot can vary slightly according to the instrument used, and the clot detection mode and instrument setting (clot detection sensitivity). Each laboratory should check and validate its own usual range, as well as target values and acceptance ranges for each new lot of controls, in its specific test conditions.

Any sample presenting an abnormal aspect (eg: lipaemic, haemolysed, partial coagulation…) should be rejected.

The **Fibriphen** reagent contains a heparin neutralizing substance.

There was no significant interference noticed for heparins UFH, LMWH, Arixtra, Hirudin, Argatroban®, Fibrin degradation products (FDP), respectively up to 2 IU/ml, 2 IU/ml, 2 µg/ml, 5 µg/ml, 2 µg/ml, 130 µg/ml added to plasma.

13. General information:

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 stabilised by activated factor XIII in presence of calcium. Fibrinogen is lysed by plasmin to fragments X and Y, first, then D and E. (1,3)

14. Indicative performances and Assay variations:

The indicative clotting times observed for this assay are in the range 4 - 7 seconds, and about 22 ± 5 seconds, respectively for the 12g/L or 3 g/L Fibrinogen concentrations, using the water bath or STAR method.

Indicative performances obtained in these conditions are as follows:

- Dynamic range: 1–12 g/L (for a sample assayed at the 1:20 dilution)

  Note: For a better accuracy, samples measured ≤ 1 g/L can be tested at the 1:10 dilution, and obtained results divided by 2; for samples measured >12g/L, the 1:40 dilution can be used and obtained results multiplied by 2.

- Accuracy: as an example, the following results were obtained using the STA-R instrument:

	Fng (g/L	N	Intra assay CV (%)	Inter assay CV (%)
Sample 1	2.67	10	2.3%	2.3%
Sample 2	1.47	10	2.6%	3.7%