Block 5, Street 9 inside The Ismailia free zone. Ismailia- Egypt
Post Code-41511
Tel.-Fax: +202 21813500/ +202 21813600
Mob.: +2 01211550941/ +2 01119225860
E-mail: sales@arenabioscientific.com
Website: www.arenabioscien.com

FIBRINOGEN-LYO

Diagnostic reagent for the in-vitro quantitative determination of plasma Fibrinogen using Clauss Clotting Time method in both automatic and manual systems.

REF: BS.1/FB01.020.0020 20 tests

CLINICAL SIGNIFICANCE

Fibrinogen is the principle plasma protein affecting the sedimentation rate. Fibrinogen concentration raises several folds during inflammation or tissue necrosis. Oestrogen ingestion, diabetes, obesity or pregnancy may also induce increased levels. Evidence as shown that plasma levels above the reference range constitute a significant independent risk factor for both coronary artery and cerebrovascular diseases.
A decreased fibrinogen level in plasma is generally associated with a disturbance of liver metabolism (cirrhosis, icterus…) or with fibrinolysis and DIC (disseminated intravascular coagulation).

METHOD PRINCIPLE

The fibrinogen level is directly proportional to the thrombin clotting time. Excess Bovine Thrombin is used to clot diluted plasma. First, a standard curve is prepared using reference plasma of known fibrinogen content at dilutions of 1/5 and 1/10. When Thrombin is added, the clotting time obtained. Next, patient plasma, at a dilution of 1/10, is clotted with Thrombin. and the resultant clotting time is used to interpolate Fibrinogen level from the standard curve.

REAGENT COMPOSITION

R1:Thrombin Reagent	lyophilized Bovine Thrombin 50 NIH* units.
R2:Buffer	Owren's buffer**,** ready to use (pH 7.35).
R3:*Fibrinogen calibrator*	lyophilized preparation of human plasma, concentration stated on the vial label.

*NIH(The National Institute of Health): The amount of thrombin which will clot 1.0 ml of a standardized fibrinogen solution in 15±0.5 sec. at 28±1.0°. Equivalent to 1.1 to 1.3 IU of thrombin.

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person.
Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the Fibrinogen reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

BioScien Fibrinogen reagent is stable until expiration date stated on label when properly stored refrigerated at 2-8°C (do not freeze).

Bovine Thrombin: reconstitute individual vials with the volume of distilled water stated on the vial label and mix.
Once reconstituted vial is stable up to 15 days when stored at 2-8°C and for 4 hours at room temperature.

Buffer: Open vial is stable up to 30 days when stored at 2-8°C.

Calibrator: reconstitute individual vials with the volume of distilled water stated on the vial label and mix for 2 minutes.
Reconstituted calibrator is stable for 2 hours at room temperature**,** 6 hours at 2-8°C.

SPECIMEN COLLECTION AND PRESERVATION

Plasma
Patient should be careful venipuncture and the blood should be transferred into anticoagulated tube containing trisodium citrate (0.11 mol/L, 3.2%). Mix blood with anticoagulant with definite ratio 9:1, for occasional patients with hematocrit less than 20% or greater than 55%, this ratio must be readjusted to ensure valid result. avoid foam formation during mixing. centrifuge immediately for 15 minutes at 1500 – 3000 rpm (approximately 1500g) on laboratory centrifuge and transfer the plasma into a clean test tube. It should be ensured that the plasma is free from platelets (PRP). Cap the test tubes to prevent deterioration of samples. Plasma must be tested immediately. However if the specimens are held at 2 - 4 °C then they may be tested within 3 hours.

SYSTEM PARAMETERS

Refer to "***Tridema BioScien***" instrument instructions for test determination parameters or equivalent.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes and automatic pipet
- Centrifuge and water bath

ASSAY PROCEDURE

Automated instrument procedure

a- Prewarm the instrument to stable state.

b- Prepare the ref. plasma and use according to the insert.

c- Prepare the FIB calibration curve:

1.Dilute the plasma as the table below

Test tube no.	I	II	III
Owren's buffer	NIL	800 µl	900 µl
Fibrinogen calibrator	200 µl	200 µl	100 µl
Dilution(calibrator)	NIL	1:5	1:10

2. Pipette 200 µl of each fibrinogen calibrator dilution into clean test tubes and prewarm for 3 minutes at 37°C.
3. Add 100 µl of reconstituted thrombin reagent (prewarmed at 37 °C for one minute) and simultaneously start stopwatch.
4. Stop the stopwatch at the first appearance of the fibrin web, as the gel clot begins to form and record the time in seconds.
5. Repeat steps 2-4 for a duplicate test on each calibrator dilution.
6. Plot the average of the duplicate test values, connect the points, which should produce a straight line.
7. The calibration curve may be extended beyond the lowest and highest point.

d- Determination of Fibrinogen concentration in sample:

1. Prepare a 1:10 dilution of plasma specimen with Owren's buffer solution.
2. To test tube at 37°C add 200 µl of 1:10 dilution of plasma sample to be tested, incubate for three minutes.
3. To the test tube add 100 µl of thrombin reagent (prewarmed at 37°C for one minute) and start the stopwatch simultaneously.
4. Stop the stopwatch at the first appearance of the fibrin web, as the gel clot begins to form and record the time in seconds.
5. Repeat steps 1-4 for a duplicate test.
6. Calculate the mean clotting time for the plasma specimen.

-If the obtained fibrinogen concentration is > 600 mg/dl, repeat the test at 1:20 dilution of the sample. The results read of the graph will be multiplied by a factor 2 for deriving the fibrinogen concentration in the sample.

- If the obtained fibrinogen concentration is <80 mg/dl, repeat the test at 1:5 dilution of the sample. The results read of the graph will be divided by a factor 2 for deriving the fibrinogen concentration in the sample.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact ***BioScien*** technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	240	240	240	240
Mean sec	208.6	706.8	208.6	706.8
SD. sec	3.0	11.9	3.2	13.9
CV. %	1.0	2.0	1.0	2.0

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)

Result obtained from ***BioScien*** Fibrinogen reagent compared with commercial reagent of the same methodology performed on more than 30 human plasmas give a correlation of 0.997.

INTERFERING SUBSTANCES

Icteric, Lipemic and hemolytic samples can modify the results in an unforeseeable way.

EXPECTED VALUES

150 - 400 mg/dl

REMARKS

- It is recommended that controls with known factor activity should be run simultaneously with each test series to validate test run.
- Incorrect mixture of blood and tri-sodium citrate, insufficient pre-warming of plasma and reagent, contaminated reagents, glassware etc. are potential source of errors.
- Oxalated plasma may induce prolonged clotting times.
- Since the Fibrinogen test functions correctly only at 37±0.5ºC temperature of all equipment's must be calibrated daily.
- The Fibrinogen may be shortened during acute inflammatory conditions which are accompanied by increase in fibrinogen levels and also by agent such as antihistamines, butabarbital, phenobarbital, caffeine, oral contraceptives and vitamin K. the Fibrinogen may be prolonged by corticosteroids, EDTA, asparaginase, clofibrate, ethanol, tetracycline, aspirin and anticoagulants such as heparin and warfarin.

REFERENCES

1. Goguel A, F.- Feuillets de Biologie, (1985), 36, (146) p.25-28.
2. Houbouyan-Reveillard et al. Spectra biologie, (2003) vol. 22, nº132 p. 33-37.
3. Biggs R. and R.G. McFarlane: Human Coagulation and its disorders, Blackwell Scientific Publications, Oxford,1962
4. Quick A.J., Hemorrhagic disease and thrombosis, 2nd Ed., Philadelphia, Lee and Fibiger, 1966.
5. Clinical Guide to Laboratory Test,4th ED., N.W. TEITZ (2006) p.928-929
6. YOUNG D.S., Effect of Drugs on Clinical laboratory Tests,4th ED (1995) p,3-513 and 3-517.
7. W.H.O. Expert Committee on Biological standardization, No. 687, 1983.

SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

EC REP

Medical Device Safety Service
MDSS GmbH
Schiffgr aben 41
30175 Hannover, Germany

CE