Fibrinogen

VonClauss method

REF HC00300 **8 x 2 mL**

Store at 2 - 8°C

CE IVD

Fibrinogen dosage in plasma

Intended use
The Fibrinogen reagent is intended for the determination of fibrinogen levels in human plasma.
For *in vitro* diagnostic use only.
For professional use only.

Clinical significance:
Fibrinogen (Factor I) is the substance in blood that forms a clot. Its determination is used to evaluate abnormal blood clotting.
Fibrinogen concentration values out of the referring elapse have been observed in acute inflammations and in pregnancy (high values); in the thrombolitic therapy, in the hepatic disease, in the congenital non fibrinogen, in DIC and in pancreatitis (low values).

Principle:
In the presence of an excess of thrombin, fibrinogen is transformed into fibrin and clot formation time is inversely proportional to the concentration of fibrinogen in the sample plasma.

Reagent Composition:

Reagent 1	Human alpha thrombin Buffered medium with calcium and preservative Lyophilised
Reagent 2	Imidazole Buffered medium with preservative

Preparation:
Reagent 1: Bring the vial at room temperature. Add 2 ml of distilled water to a vial of lyophilized thrombin. Let stand for 5 minutes before swirling the vial gently in upright position a few times to mix it. Prevent contact of the fluid with the stopper. Keep the thrombin at room temperature (20-25 °C) for at least 30 minutes until complete reconstitution.
Just before use, swirl the vial gently 5-10 times in upright position. Do not shake.
Reagent 2: Ready-to-use.

Storage and stability
All the components of the kit are stable at 2-8 °C up to the date of expiration as specified. Do not freeze!
Stability of reagent 1 after reconstitution: 7 days at 2-19 °C and 3 days at 20-25 °C in the original vial.
Stability of reagent 2 after opening: 8 weeks at 2-8 °C, 2 weeks at 20-25 °C in the original vial.

Additional material required, not provided
- Optical or mechanical coagulation reader
- General laboratory equipment

Precautions
1. Standard guidelines for handling infectious agents and chemical reagents should be observed throughout all procedures. All reagents and contaminated waste such as patient samples and used material should be properly disposed of in accordance to the relevant national regulations.
2. Do not use the reagent beyond the expiration date printed on the label.
3. Avoid microbial contamination of the reagent or erroneous results may occur.
4. Each donor unit used in the preparation of this reagent is tested and found to be negative for the following tests: antibodies to HIV, hepatitis C and hepatitis B surface antigen. However, the product must be handled with care, observing the precautions recommended for biohazardous material.
5. Reagent 2: Danger. H360: May damage fertility or the unborn child. P280: Wear eye protection, face protection, protective clothing, protective gloves. P501: Dispose of contents in an appropriate container observing applicable local regulations.

Samples:
SAMPLE: Plasma obtained from whole blood anti-coagulated with 3,2% (109 mmol/L) sodium citrate. The use of higher concentrations of sodium citrate (3,8%, 129 mmol/L) is not recommended.
SAMPLE COLLECTION: Immediately add nine parts of freshly collected whole blood to one part of anticoagulant.
SAMPLE PREPARATION: Mix the blood carefully and centrifuge the sample to obtain the plasma, place it in a test tube, keep at room temperature (20-25 °C) and perform the measurement within 4 hours. Do not store the sample at 2-8 °C. Refer to the Clinical and Laboratory Standards Institute (CLSI) guidelines H21-A5.

Test procedure:
Each sample should be tested at least twice.
1. Bring reagents 1 and 2 at room temperature.
2. Dilute the patient plasma 1/10 in buffer (reagent 2).
3. Transfer in a cuvette:

Diluted sample	200 µL
Incubate for 2 minutes at 37 °C Gently swirl the vial with Thrombin reagent (reagent 1) just before use and add abruptly:	
R.1. Thrombin	100 µL
Start the timer immediately. Measure time of clot formation.	

If using an instrument to perform this test, refer to the appropriate Instrument Operator's Manual for detailed instructions.

Results
The result can be reported in g/L.
- The fibrinogen value can be obtained by simple reading of the tables that are included in the kit.
- The fibrinogen value can also be calculated from the calibration curve, obtained as described below.

In case the obtained patient values are lower than 1 g/L it is recommended to retest the plasma at a 1/5 dilution.
In case the obtained patient values are higher than 5 g/L it is recommended to retest the plasma at a 1/20 dilution.

Calibration
Use a calibrator (HC00600) to make dilutions in Imidazole (reagent 2) as described in the table below. Separately prepare dilution 1/7. Thereafter, dilutions 1/10, and the 1/20 and 1/30 have to be generated by serial dilution. The diluted calibrator must be processed within 2 hours.

Dilution	1/7	1/10	1/20	1/30
Calibrator (mL)	0,1 (-)**	0,2 (-)**	1,0 (1/10)**	1,0 (1/20)**
Imidazole (mL)	0,6	1,8	1,0	0,5
Factor (F)	10/7	10/10	10/20	10/30
Concentration (g/L)	C* x F	C* x F	C* x F	C* x F

* Calibrator concentration; ** Dilution of calibrator solution that has to be used

Calculate the mean of the duplicate clotting times. Construct a log-log curve that plots the fibrinogen concentrations (g/L) of the different dilutions versus the clotting time (sec). Draw the straight line of best fit.

Expected values:
Normal values are between 2,0 and 4,0 g/L.
Each laboratory should determine its own reference range.

Quality control
Normal and pathological controls (HC00500) are recommended for verifying the measurement. Each laboratory should establish its own quality control program.

Cypress Diagnostics: Nijverheidsstraat 8 • 2235 Hulshout • Belgium
www.diagnostics.be • Tel: ++ 32 15 67 67 68 • e-mail: cypress@diagnostics.be

Limitations

1. The included tables are method specific (type of analyzer, laboratory practice and conditions). In case of deviating results, the laboratory should construct its own calibration curve as described above.
2. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.
3. The results obtained for Fibrinogen may be influenced by drugs and other pre-analytical interfering agents.

Specifications for use on CYANCoag 2Ch

Linearity: 1,4 – 5 g/L

- In case the obtained patient or control value is lower than or equal to 1,4 g/L OR if the coagulation time is higher than 20 seconds it is recommended to retest the plasma at a 1/5 dilution and divide the obtained concentration value by 2.
- In case the obtained patient or control values are higher than 5 g/L it is recommended to retest the plasma at a 1/20 dilution and multiply the obtained concentration value by 2.

Calibration

Use a calibrator (HC00600) to make dilutions in Imidazole (reagent 2) as described in the table below. Separately prepare dilution 1/7. Thereafter, dilutions 1/10, and the 1/12,5 and 1/15 have to be generated by serial dilution.

The diluted calibrator must be processed within 2 hours.

Dilution	1/7	1/10	1/12,5	1/15
Calibrator (mL)	0,1 (-)**	0,2 (-)**	1,2 (1/10)**	0,75 (1/12,5)**
Imidazole (mL)	0,6	1,8	0,3	0,15
Factor (F)	10/7	10/10	10/12,5	10/15
Concentration (g/L)	C* x F	C* x F	C* x F	C* x F

*** Calibrator concentration; ** Dilution of calibrator solution that has to be used**

Calculate the mean of the duplicate clotting times. Insert the calibration points in the CYANCoag 2CH analyzer. Omit calibration points that have a coagulation time of more than 20 seconds.

Bibliography

1. CLSI: Collection, transport, and Processing of Blood Specimens for Testing plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline – Fifth Edition. CLSI document H21-A5; 28:5; 2008.
2. CLSI: Procedure for the Determination of Fibrinogen in Plasma; Approved Guideline- Second Edition. CLSI document: H30-A2; 21:18; 2001.
3. Clauss A: Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens. Acta Haematol; 17:237-46; 1957

09.2021, Rev. 5.0

Cypress Diagnostics: Nijverheidsstraat 8 • 2235 Hulshout • Belgium
www.diagnostics.be • Tel: ++ 32 15 67 67 68 • e-mail: cypress@diagnostics.be

Fibrinogen
Fibrinogène
VonClauss method

Code: HC00300 8 x 2 ml

Calibration Curve Values Valeurs Courbe d'étalonnage	
Lot 910418A	
CYANCoag line - Mechanical Readers Ligne CYANCoag - Lecteurs Mécaniques	
Sample dilution Dilution échantillon	1/10
Concentration (g/l)	Sec
4	8,3
3	10,1
2	14,3
1	29,9
Sysmex CA line - Optical Readers Ligne Sysmex CA - Lecteurs Optiques	
Sample dilution Dilution échantillon	1/10
Concentration (g/l)	Sec
4	9,2
3	11,1
2	14,7
1	26,1
Stago line - Mechanical Readers Ligne Stago - Lecteurs Mécaniques	
Sample dilution Dilution échantillon	1/20
Concentration (g/l)	Sec
4	15,6
3	20,1
2	28,9
1	53,7

Cypress Diagnostics: Nijverheidsstraat 8 • 2235 Hulshout • Belgium
www.diagnostics.be • Tel: ++ 32 15 67 67 68 • e-mail: cypress@diagnostics.be

Fibrinogen
Fibrinogène
VonClauss method

Code: HC00300 8 x 2 ml

CYANCoag line - Mechanical Readers Ligne CYANCoag - Lecteurs Mécaniques	
Sample dilution Dilution échantillon	1/10
Lot 910418A	
Sec	Concentration (g/l)
5,0	6,5
5,5	5,9
6,0	5,3
6,5	4,9
7,0	4,5
7,5	4,2
8,0	3,9
8,5	3,7
9,0	3,5
9,5	3,3
10,0	3,1
10,5	2,9
11,0	2,8
11,5	2,7
12,0	2,6
12,5	2,5
13,0	2,4
13,5	2,3
14,0	2,2
14,5	2,1
15,0	2,0
15,5	2,0
16,0	1,9
16,5	1,8
17,0	1,8
17,5	1,7
18,0	1,7
18,5	1,6
19,0	1,6
19,5	1,5
20,0	1,5
20,5	1,4
21,0	1,4
21,5	1,4
22,0	1,3
22,5	1,3
23,0	1,3
23,5	1,3
24,0	1,2
24,5	1,2
25,0	1,2
25,5	1,1
26,0	1,1
26,5	1,1
27,0	1,1
27,5	1,1
28,0	1,0
28,5	1,0
29,0	1,0

Sysmex CA line - Optical Readers Ligne Sysmex CA - Lecteurs Optiques	
Sample dilution Dilution échantillon	1/10
Lot 910418A	
Sec	Concentration (g/l)
5,0	8,7
5,5	7,6
6,0	6,8
6,5	6,1
7,0	5,6
7,5	5,1
8,0	4,7
8,5	4,3
9,0	4,0
9,5	3,7
10,0	3,5
10,5	3,3
11,0	3,1
11,5	2,9
12,0	2,7
12,5	2,6
13,0	2,5
13,5	2,3
14,0	2,2
14,5	2,1
15,0	2,0
15,5	1,9
16,0	1,9
16,5	1,8
17,0	1,7
17,5	1,7
18,0	1,6
18,5	1,5
19,0	1,5
19,5	1,4
20,0	1,4
20,5	1,3
21,0	1,3
21,5	1,3
22,0	1,2
22,5	1,2
23,0	1,2
23,5	1,1
24,0	1,1
24,5	1,1
25,0	1,0
25,5	1,0
26,0	1,0
26,5	1,0
27,0	0,9
27,5	0,9
28,0	0,9
28,5	0,9
29,0	0,9

Stago line - Mechanical Readers Ligne Stago - Lecteurs Mécaniques	
Sample dilution Dilution échantillon	1/20
Lot 910418A	
Sec	Concentration (g/l)
10,0	6,6
11,0	5,9
12,0	5,4
13,0	4,9
14,0	4,5
15,0	4,2
16,0	3,9
17,0	3,6
18,0	3,4
19,0	3,2
20,0	3,0
21,0	2,9
22,0	2,7
23,0	2,6
24,0	2,5
25,0	2,4
26,0	2,3
27,0	2,2
28,0	2,1
29,0	2,0
30,0	1,9
31,0	1,8
32,0	1,8
33,0	1,7
34,0	1,7
35,0	1,6
36,0	1,6
37,0	1,5
38,0	1,5
39,0	1,4
40,0	1,4
42,0	1,3
44,0	1,2
46,0	1,2
48,0	1,1
50,0	1,1
52,0	1,0
54,0	1,0
56,0	1,0
58,0	0,9
60,0	0,9
62,0	0,9
64,0	0,8
66,0	0,8
68,0	0,8
70,0	0,7
72,0	0,7
74,0	0,7
76,0	0,7

Cypress Diagnostics: Nijverheidsstraat 8 • 2235 Hulshout • Belgium
www.diagnostics.be • Tel: ++ 32 15 67 67 68 • e-mail: cypress@diagnostics.be