

PRODUCT CODE
CR002
INTENDED USE

BioClot-APTT reagent is intended for partial Thromboplastin (APTT) determination using ellagic acid, as an activator.

CLINICAL SIGNIFICANCE

The activated partial thromboplastin time (APTT) is used as a general screening test for the detection of coagulation abnormalities in the intrinsic pathway.

Activated Partial Thromboplastin Time is prolonged by a deficiency of coagulation factors of the intrinsic pathway of the human coagulation mechanism such as factor XII, XI, IX, VIII, X, V, II and Fibrinogen.

Determination of APTT helps in estimating abnormality in most of the clotting factors of the intrinsic pathway including congenital deficiency of factor VIII, IX, XI and XII and is also a sensitive procedure for generating heparin response curves for monitoring heparin therapy.

PRINCIPLE

In presence of Calcium ions cephaloplastin reagent activates coagulation factors of intrinsic pathway in plasma leading to clot formation.

APTT is prolonged by a deficiency of one or more of these clotting factors of the intrinsic pathway and in the presence of coagulation inhibitors like heparin.

REAGENTS

REAGENT 1: BioClot-APTT reagent is a liquid ready to use activated cephaloplastin reagent for the determination of Activated Partial Thromboplastin Time. It is a phospholipids preparation derived from rabbit brain with ellagic acid as an activator.

Each batch of the reagent undergoes rigorous quality control at various stages of manufacture for its sensitivity and performance.

REAGENT 2: calcium chloride Solution (0.025mol/l)

ADDITIONAL REQUIREMENTS

(1) Glass test tubes. (2) Precision pipettes. (3) Stop watch. (4) Water bath or heating block at 37°C. (5) Fresh Normal Pooled Plasma (FNP). (6) CaCl₂ (0.025 mol/l).

STORAGE AND STABILITY

Store tightly closed at 2-8°C, **Do Not Freeze**. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. Once opened the reagent is stable for 3 months at 2-8°C, 1 week at 18-25°C, and 2 days at 37°C provided it is not contaminated and capped tightly when not in use.

PRECAUTIONS

The reagent is for "in vitro" diagnostic use. Avoid exposure of the reagent to elevated temperature and contamination. Immediately replace and recap after use and store at recommended temperature homogenize each time it is used. All patient samples should be handled as if they were capable of transmitting infection. All reagents and samples must be discarded according to the local regulations in force.

SPECIMEN AND SAMPLE PREPARATION

No special preparation of the patient is required prior to sample collection by approved techniques. Withdraw blood without undue venous stasis and without frothing into a plastic syringe fitted with a short needle of 19 to 20 SWG. The venipuncture must be a 'clean' one and, if there is any difficulty, take a new syringe and needle and try another vein. Transfer the blood into tubes, after detaching the needle from the syringe.

Mix exactly nine parts of freshly collected blood with one part of tri-sodium citrate (0.11 mol/l, 3.2%). Centrifuge immediately for 15 minutes at 1500 RPM and transfer the plasma into a clean test tube. Plasma must be tested within three hours of blood collection. For heparin determination, platelet deficient plasma should be used; hence higher centrifugation time is required.

FNP COLLECTION

Prepare a plasma pool (FNP) of freshly collected blood from at least five normal healthy donors and process as above. Plasma must be tested within three hours of blood collection.

TEST PROCEDURE
Manual Method

- Before use, the reagent should be mixed well by gentle swirling. Do not shake.
- Aspirate from the reagent vial enough reagent for the immediate testing requirement in a thoroughly clean and dry test tube. Bring this reagent to room temperature before prewarming at 37°C for testing purposes.
- Separate test tubes containing BioClot-APTT Reagent and Calcium Chloride Solution should be brought to 37°C. (Depending on volume, approximately 5 to 10 minutes required). Do not incubate the test plasma.
- In glass test tube, add 100µl test plasma and 100µl BioClot-APTT Reagent. Shake tube briefly to mix the reagent and plasma, place tube at 37°C for 3 to 5 minutes.
- Following incubation period, add forcibly 100µl of prewarmed calcium chloride into the plasma and BioClot-APTT mixture, simultaneously start a stopwatch. Shake tube briefly to mix contents, keep at 37°C for 15 seconds.
- Following 15 seconds incubation, remove the tube, gently tilt back and forth until a gel /clot forms, stop the watch and record time in seconds.
- Repeat steps 4-6 for a duplicate test using the same test plasma.
- Find the average from the duplicate test values. This is the Activated Partial Thromboplastin Time (APTT of patient plasma).
- Similarly repeat steps 4-6 twice, and record duplicate values using FNP in place of test plasma (APTT of FNP).

If a coagulation instrument is being used to perform the tests, the instrument manufacturer's instructions must be strictly adhered to.

Calibration Curve Method (For determination of heparin concentration)

- Dilute heparin (as used for treatment) with physiological saline to a concentration of 10 U/ml.
- Mix 200µl of 10 U/ml diluted heparin with 1.8 ml of FNP to give a heparin standard of 1 U/ml concentration.
- Dilute the heparin standard as prepared above (1U/ml) with FNP as follows:

Test tube number	1	2	3	4	5	6	7
Heparin std.(1IU/mL)	500	400	300	200	100	100	-
FNP in µL	-	100	200	300	400	900	500
Heparin Concentration(IU/mL)	1.0	0.8	0.6	0.4	0.2	0.1	0.0

- Pipette 100µl each of the seven heparin dilutions into clean test tubes.
- Add 100µl BioClot-APTT reagent to each test tube.
- Mix well and incubate each test tube at 37°C for exactly 3 minutes before testing.
- Forcibly add 100µl calcium chloride (prewarmed at 37°C) to each

8. Gently tilt the tube back and forth and stop the stopwatch as the first fibrin strand is visible and the gel / clot formation begins. Record the time in seconds.
9. Repeat steps 4-8 for each dilution for duplicate test, and find the average of the duplicate test values.
10. Plot the mean of the double determination in 'seconds', against each heparin concentration using **APTT** graph paper.
11. Clotting times (APTT) of test specimens can be interpolated against the heparin concentration to determine the heparin concentration of the sample in U/ml.

CALCULATION
Manual Method

- a. The results may be reported directly in terms of the mean of the double determination of the APTT of the test plasma clotting time. It is suggested that the results be reported to the clinicians in conjunction with the normal range.

OR

- b. As ratio as follows,

$$R = \frac{\text{APTT patient plasma (in seconds)}}{\text{APTT of FNP (in seconds)}}$$

- c. Calibration Curve Method

Heparin concentration in the test sample can be directly obtained from the APTT-E calibration curve by interpolating the test plasma clotting time against heparin concentration in U/ml.

EXPECTED VALUES

Normal values are between 22-35 seconds.

Reference values for healthy individuals may vary from laboratory to laboratory depending on techniques and instrumentation used.

REMARKS

1. Due to inter and intra laboratory variations users must establish their own normal population range as well as normal and abnormal range.
2. It is recommended that controls with known factor activity should be run simultaneously with each test series routinely.
3. Incorrect mixture of blood and tri-sodium citrate, insufficient prewarming of plasma and reagent, contaminated reagents, glassware etc. are potential source of errors.
4. Incorrect dilution of heparin is also a potential source of error.
5. Oxalated plasma may induce prolonged clotting times.
6. Clotting time of patients on anticoagulant therapy depends upon the type and dosage of anticoagulant and also the time lag between the specimen collected and the last dose.
7. Abnormalities of coagulation factor VII, factor XIII and platelets are not detected by this test procedure.
8. For automated equipment it is strongly recommended that the equipment manufacturer's methodology be strictly adhered to.
9. In heparin monitoring time of collection of blood sample is important since the in-vivo half-life of heparin is approximately 1.5 hours. When it is administered intravenously it has an immediate anti-coagulant effect but its efficacy decreases rapidly with time.
10. Platelet factor IV, a heparin-neutralising factor can be released due to platelet aggregation or damage. In order to prevent this phenomenon in-vitro the specimen should be collected with a minimum of trauma.

11. Decrease in APTT time is observed in males under estrogen therapy and oral contraceptive administration in females.

PERFORMANCE CHARACTERISTICS
Heparin Sensitivity

The sensitivity of **BioClot APTT** to heparin was determined by adding known amounts of heparin to pooled normal plasma and performing the APTT. The following results were obtained on a coagulometer based on turbodensitometric method of clot detection with one Lot of **APTT**

Heparin Concentrate (U/mL)	APTT (seconds)
0.0	25.3
0.1	31.4
0.2	40.2
0.4	52.0
0.6	80.3
0.8	120.8
1.0	158.3

Each laboratory must establish its own heparin calibration curve using the same source of heparin used for therapy in that institution. Variations can result from different brands of heparin, tissue origin and salt forms.

Factor Sensitivity














An APTT reagent with good sensitivity must demonstrate a prolonged clotting time in samples having 30-40% factor VIII and factor IX activity. **APTT-E** was evaluated by diluting a normal pooled plasma with factor deficient plasma and measuring the clotting time.

Sensitivity to Factor VIII		Sensitivity to Factor IX	
Activity of Factor (%)	APTT (seconds)	Activity of Factor (%)	APTT (seconds)
100	33.7	100	43.8
50	42.0	50	47.5
25	46.8	25	52.0
12.50	50.0	12.50	56.8
6.25	62.4	6.25	60.0
3.12	67.0	3.12	68.0
1.50	73.6	1.50	70

INTERFERENCES

Do not use sodium oxalate, EDTA or heparin as anticoagulant. Oral contraceptives, estrogens or pregnancy interfere in the assay.

SYMBOL ON LABELS

Symbol	Signify	Symbol	Signify
	Catalogue Number		Pack Size
	Expiry Date		Volume
	Storage Condition		Lot Number
	Instruction for Use		In Vitro Diagnostics
	Manufacturing Date		Manufacturer
	Number of Tests		For Single Use Only
	EC Representative		

REFERENCES

1. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999
2. Biggs, R., ed: Human Blood Coagulation, Haemostasis and Thrombosis, Blackwell Scientific Publications Oxford, England,

- 1972
3. NCCLS guideline H21-A3, Vol. 18, No. 20.
 4. CRC, Handbook Series in Clinical Laboratory, Science, Section 1: Haematology, Volume III, 1980. CRC Press, Inc. Boca Raton, Florida.