APTT

2 x 4 mL
52602001

Intended Use
This reagent is intended for the *in vitro* determination of Activated Partial Thromboplastin Time (APTT) in citrated plasma.

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. APTT is sensitive to deficiencies or abnormalities of factors VIII, IX, XI, XII, X, V, II and I. APTT is also sensitive to inhibitors of blood coagulation such as lupus inhibitor and fibrin/fibrinogen degradation products. APTT is the most widely used method for monitoring intravenous heparin anticoagulation therapy.

Principle
In presence of Calcium ions cephaloplastin activates coagulation factors of intrinsic pathway in plasma leading to clot formation. Clotting time is proportional to the concentration of factors VIII, IX, XI and XII as well as common pathway factors II, V, and X. As the reagent is prepared using one single species rabbit brain, it has the required sensitivity to be used in heparin assays, also has better sensitivity for factors VIII & LA.

Kit Components

Reagent/ Component	Product Code 52602001	Description
APTT Reagent 1	2 x 4 mL	Calcium chloride solution 0.020 M/L
APTT Reagent 2	2 x 4 mL	Rabbit brain cephalin Ellagic acid activator Buffer Stabilizers and preservatives
Tri sodium citrate	1 x 10 mL	Tri sodium citrate (0.109 M/L) 3.2%

Risk & Safety
Material Safety data sheets (MSDS) will be provided on request.

Reagent Storage and Stability
The sealed reagents are stable upto the expiry date stated on the label, when stored at 2 - 8°C.

Precaution
- Venous blood should be directly drawn into the tube containing anticoagulant.
- Ensure that the sample is free of microclots.
- Separate plasma immediately by centrifugation after collection of blood.
- The test should be done preferably within two hours of blood collection.
- Plasma must be stored in siliconized glass tubes or plastic containers.
- Avoid turbid, lipemic or hemolyzed samples.
- Use clean dry micropipette tips and plastic ware to dispense the reagent.
- Mix the reagent (by gentle swirling) before use.
- Close reagent vial and replace immediately to 2-8°C after dispensing.

Waste Management
Reagents must be disposed off in accordance with local regulations.

Sample
Citrated plasma

Materials provided
APTT Reagent 1, APTT Reagent 2 and Tri sodium citrate

Materials Required but Not Provided
- Pipettes& Tips
- Test Tubes & racks
- Timer
- Incubator
- Analyzer

Sample Collection & Preparation
Citrated plasma.

Mix gently, 9 parts of blood in a plastic tube or siliconzied glass tube containing 1 part of 3.2% tri-sodium citrate solution (0.109 M/L). Centrifuge immediately for 15 minutes at 3000 rpm to obtain platelet poor plasma. Transfer supernatant plasma in a siliconized glass tube or plastic tube immediately; do not disturb the buffy coat while collecting supernatant plasma. The test should be done preferably within 2 hours of blood collection.

Procedure
APTT of each sample should be determined at each level twice. This procedure pertains to manual or semi-automated coagulation systems. Refer to your instrument manual for more detailed instrument specific instructions.

Bring contents of the vial to room temperature and then swirl gently to mix to a homogenized suspension. Keep the reagents at 37°C for 10 minutes prior to use.

1. Gently swirl the reagent vials before use. Do not shake.
2. Pre-warm enough volume of Reagent 1 ($CaCl_2$) for immediate use, in a clean & dry plastic test tube at 37°C.
3. Pipette 100 µL of test plasma or control in to a test cuvette at 37°C.
4. Pipette 100 µL of the pre-warmed reagent 2 (APTT Reagent) in to the test cuvette.
5. Mix well and incubate at 37°C for 3 minutes.
6. Forcibly pipette 100 µL of pre warmed Reagent1 ($CaCl_2$) into the test cuvette.
7. Start a timer simultaneously and record the clotting time in seconds.

Calibration curve for the determination of Heparin concentration:

Dilute Heparin (as used for treatment) with physiological saline to concentration of 10 IU/mL.

Mix 0.2 mL of 10 IU/mL diluted heparin with 1.8 mL of FNP (Fresh Normal Plasma) to yield heparin standard of 1 IU/mL concentration.

Dilute the Heparin standard as prepared above (1 IU/ml) with FNP as follows.

Test Tube	1	2	3	4	5	6	7
Heparin std.(1IU/mL)	0.5	0.4	0.3	0.2	0.1	0.1	-
FNP in mL	-	0.1	0.2	0.3	0.4	0.9	0.5
Heparin con.(IU/mL)	1	0.8	0.6	0.4	0.2	0.1	0

Procedure: Manual method to estimate Heparin concentration in the plasma/ sample.

. Pipette 0.1mL of each Heparin dilution into clean test tubes.
. Add 0.1 mL APTT Reagent (pre-warmed at 37°C). Mix and incubate for exactly 3 minutes at 37°C.
. Add. 0.1 mL Pre-warmed Calcium chloride (0.0290 M/L) and simultaneously start stopwatch.
. Observe clot formation carefully and note the time at the appearance of first fibrin web.
. Plot mean of double determination in seconds against each heparin concentration on heparin calibration graph paper provided.
. Connect points in a straight line.

*Plot clotting time of sample on the calibration curve and read heparin concentration in IU/mL

Note: Laboratories using coagulometers should follow instructions (sequence) of the coagulometer manufacturer.

Warranty: The product is designed to perform as described in the pack insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Calculation
The result of APTT test can be reported directly in seconds.

Reference Range
The normal values are between 21-38 seconds (at 3 minutes activation). The normal time depends on the method, activation time, instrument etc. and must be determined in each laboratory. Results obtained for patient samples are to be correlated with clinical findings of patient for interpretation and diagnosis.

Bibliography

1. Dacie, J.V., Lewis, S.M.; Practical hematology. 1984
2. R Biggs, R, Mcfarlane, R. G; Human blood coagulation and its disorders 1963
3. Burtis, *et al*. Tietz: Text book of Clinical Chemistry AACC 1999
4. Roadck, B. F; Diagnostic Hematology, clinical principles and applications 2nd edition.
5. John Bernad Henry: Clinical Diagnosis and management by laboratory methods 20th edition.
6. Data on file of Agappe Diagnostics Ltd. Kerala.

SYMBOLS USED ON THE LABELS

SYMBOLS USED ON THE LABELS: IVD IN VITRO DIAGNOSTIC USE | SEE PACKAGE INSERT FOR PROCEDURE | LOT LOT NUMBER | MANUFACTURER'S ADDRESS | MANUFACTURING DATE | EXPIRY DATE | TEMPERATURE LIMIT

BIOSS BioTechnology, GmbH
Boekhulter Weg 1a, 47638 Straelen, Germany
E-mail: sales@biossbiotech.de, support@biossbiotech.de
EC REP Arthrex GmbH
Erwin-Hielscher-Strasse 9, 81249 Munchen, Germany

ADS/V.02/060114

CE ISO 9001 : 2008 ISO 13485 : 2003

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Heparin - Calibration Curve

HEPARIN	U/ml	0	0.1	0.2	0.4	0.6	0.8	1.0
PTT	Sec.							

Lot. No : .....

Date : .....

Test performed by : .....