



SOYABEAN CEPHALOPLASTIN REAGENT FOR PARTIAL THROMBOPLASTIN TIME (APTT) DETERMINATION USING ELLAGIC ACID, AS AN ACTIVATOR

SUMMARY

The arrest of bleeding depends upon primary platelet plug formed along with the formation of a stable fibrin clot. Formation of this clot involves the sequential interaction of a series of plasma proteins in a highly ordered and complex manner and also the interaction of these complexes with blood platelets and materials released from the tissues.

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.

PRESENTATION

REF	10635123
CELIN-SE®	12 x 3 ml
Pack insert	1

REAGENT

CELIN-SE® is a liquid ready to use activated cephaloplastin reagent for the determination of Activated Partial Thromboplastin Time. It is a phospholipid preparation derived from soyabean with ellagic acid as an activator.

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

REAGENT STORAGE AND STABILITY

(a) Store the reagent at 2-8°C. **DO NOT FREEZE.** (b) The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label. The uncontaminated reagent is stable as per the **labeled shelf life at 2-8°C, 1 week at 18-25°C, 2 days at 37°C.**

PRINCIPLE

Cephaloplastin activates the coagulation factors of the intrinsic pathway of the coagulation mechanism in the presence of calcium ions. APTT is prolonged by deficiency of one or more of these clotting factors of the intrinsic pathway and in the presence of coagulation inhibitors like heparin.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. CELIN-SE® reagent is not from human source hence contamination due to HBsAg and HIV is practically excluded.
3. It is **very important** that clean and dry pipette tips be used to dispense the reagent.
4. **Avoid exposure** of the reagent to elevated temperature and contamination. **Immediately** replace cap after use and store at recommended temperatures **only**.
5. The package insert is common for CELIN-SE® System Pack, Cat. No.: 10635063.

SAMPLE COLLECTION AND 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 veinpuncture 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%) or PROFACT available from TULIP Cat. No. 10660020.

Centrifuge immediately for 15 minutes at 1500g 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 atleast five normal healthy donors and process as above. Plasma must be tested within three hours of blood collection.

SYMBOL KEYS

	Temperature limitation		Manufacturer		Batch Number/ Lot Number		Description of reagent
	Use by		Consult Instructions for use		In vitro Diagnostic Medical Device		Authorised Representative in the European Community
	Date of Manufacture		Catalogue Number		This side up		



REGD. OFFICE: GITANJALI, TULIP BLOCK, DR. ANTONIO DO REGO BAGH, ALTO SANTACRUZ, BAMBOLIM COMPLEX P.O., GOA-403202, INDIA. Website: www.tulipgroup.com
MANUFACTURING UNIT: PLOT NOS. 92/96, PHASE II C, VERNA IND. EST., VERNA, GOA-403722, INDIA.



CMC Medical Devices & Drugs S.L., C/ Horacio Lengua No. 18, CP 29006, Malaga, Spain

ADDITIONAL MATERIAL REQUIRED

(a) 12 x75 mm test tubes (b) 0.1 ml, 0.2 ml and 2.0 ml precision pipettes (c) Stopwatch (d) Waterbath or heating block at 37°C (e) Fresh Normal Pooled Plasma (f) CaCl₂ (~0.025 mol/l) available from Tulip, Cat. Nos. 10633010, 10633100.

TEST PROCEDURE

Manual Method

1. Bring reagents to room temperature. Mix the contents of the reagent vial to homogenize the suspension completely.
2. Aspirate from the reagent vial enough reagent for the immediate testing requirement in a thoroughly clean and dry test tube.
3. Separate test tubes containing CELIN-SE® 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.
4. To a 12 x 75 mm test tube add 0.1 ml test plasma and 0.1 ml CELIN-SE®. Shake tube briefly to mix the reagent and plasma, place tube at 37°C for 3 to 5 minutes.
5. Following incubation period, add forcibly 0.1ml prewarmed calcium chloride into the plasma and CELIN-SE® mixture, simultaneously start a stopwatch. Shake tube briefly to mix contents, keep at 37°C for 20 seconds.
6. Following 20 seconds incubation, remove the tube, gently tilt back and forth until a gel clot forms, stop the watch, record time.
7. Repeat steps 2-6 for a duplicate test using the same test plasma.
8. Find the average from the duplicate test values. This is the Activated Partial Thromboplastin Time (APTT of patient plasma).
9. Similarly repeat steps 2-6 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 manufacturers instructions must be strictly adhered to.

Calibration Curve Method (For determination of heparin concentration):

1. Dilute heparin (as used for treatment) with physiological saline to a concentration of 10 U/ml.
2. Mix 0.2 ml of 10 U/ml diluted heparin with 1.8 ml of FNP to give a heparin standard of 1U/ml concentration.
3. Dilute the heparin standard as prepared above (1 U/ml) with FNP as follows:

Test tube No.	1	2	3	4	5	6	7
Heparin Standard (1U/ml) in 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 Concentration (U/ ml)	1	0.8	0.6	0.4	0.2	0.1	0.0

4. Pipette 0.1 ml each of the seven heparin dilutions into clean test tubes.
5. Add 0.1 ml CELIN-SE® reagent to each test tube.
6. Mix well and incubate each test tube at 37°C for exactly 3 minutes before testing.
7. **Forcibly** add 0.1 ml calcium chloride (prewarmed at 37°C) to each test tube, one by one and simultaneously start the stopwatch.
8. Gently tilt the tube back and forth and stop the stopwatch as the first fibrin 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 CELIN-SE® 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 AND REPORTING OF RESULTS

Manual Method

(a) The result may be reported directly in terms of the mean of the double determination of the APTT of the test plasma clotting time.

OR

(b) As a ratio R as follows:

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

Calibration Curve Method

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

EXPECTED VALUES

Normal values using CELIN-SE® reagent are between 22-35 seconds at 3 minutes activation time. Between manual and Turbodensitometric instrument results a variation of 1-2 seconds maybe expected. For photo optical instruments, it is recommended that each laboratory must establish their own normal range.

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 dilutions of heparin are also a potential source of errors.
5. Oxalated plasma may induce prolonged clotting times.
6. Clotting time of patients on anticoagulant therapy depend 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 manufacturers 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-neutralizing 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.

WARRANTY

The product is designed to perform as described on the label and the package insert. The manufacturer disclaims any implied warranty of use for any purpose.

BIBLIOGRAPHY

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5. Data on file: Tulip Diagnostics (P) Ltd.