















### SYMBOL KEYS

 Temperature limitation	 Manufacturer	 This side up	 Production Site	 Prothrombin Time
 Use by	 Consult Instructions for use	 Authorised Representative in the European Community		
 Date of Manufacture	 Catalogue Number	 Reconstitute with stated amount of distilled water		
 Batch Number/ Lot Number	 In vitro Diagnostic Medical Device	 Description of reagent		



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**LYOPLASTIN**<sup>®</sup>

(ISI-1.0)

### THROMBOPLASTIN REAGENT FOR PROTHROMBIN TIME (PT) DETERMINATION

#### 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 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.

Tissue Thromboplastin, in the presence of calcium, is an activator, which initiates the extrinsic pathway of coagulation, which includes plasma coagulation factors VII, X, V, Prothrombin and Fibrinogen.

During oral anticoagulant therapy most of the Vitamin K dependent factors such as II, VII, IX, X, Protein C, and Protein S are depressed, as also during the deficiencies of clotting factor activity which may be hereditary or acquired.

Prothrombin Time determination is the preferred method for presurgical screening, as a liver function test, determination of congenital deficiency of factors II, V, VII and X and for monitoring of patients on oral anticoagulant therapy.

#### PRESENTATION

 REF	10623123	10623124
LYOPLASTIN <sup>®</sup>	12 X 3 ml	12 X 4 ml
INR conversion table	1	1
Packinsert	1	1

#### REAGENT

LYOPLASTIN<sup>®</sup> is a sensitive, **Lyophilized Calcified Thromboplastin Reagent**, which is derived from rabbit brain. 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. (c) The reconstituted LYOPLASTIN<sup>®</sup> reagent can be used for 10 days when stored at 2-8°C provided it is not contaminated. (d) It is strongly recommended that enough reconstituted reagent should be retrieved for the days use and the unused reagent should be immediately replaced to 2-8°C.

#### PRINCIPLE

Tissue Thromboplastin in the presence of calcium activates the extrinsic pathway of human blood coagulation mechanism. When LYOPLASTIN<sup>®</sup> reagent is added to normal citrated plasma, the clotting mechanism is initiated, forming a solid gel clot within a specified period of time. The time required for clot formation would be prolonged if there is acquired or congenital deficiency of factors/ factor activity in the extrinsic pathway of the coagulation mechanism or reduction in the activity of Vitamin K dependent clotting factors during oral anticoagulant therapy.

#### NOTE

- In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
- LYOPLASTIN<sup>®</sup> reagent is not from human source hence contamination due to HBsAg and HIV is practically excluded.
- Reagent contains 0.01% Thimerosal as preservative.
- It is **very important** that scrupulously clean and dry micropipette tips be used to aspirate / dispense the reagent.
- Avoid exposure** of the reconstituted LYOPLASTIN<sup>®</sup> reagent to elevated temperatures, contamination and undue stress due to high and low temperature exposure cycles. **Immediately** replace reagent cap after use and store at recommended temperatures **only**.
- Do not use damaged or leaking reagents.

#### ADDITIONAL MATERIAL REQUIRED

12 x 75 mm test tubes (plastic tubes are preferred), 0.1 ml and 0.2 ml precision pipettes, 1 ml precision pipette, distilled water, Stop watch, Water bath or heating block at 37°C, Fresh normal plasmas for establishing MNPT.

#### REAGENT PREPARATION

Bring the LYOPLASTIN<sup>®</sup> reagent to room temperature (25-30°C) prior to reconstitution. LYOPLASTIN<sup>®</sup> reagent is reconstituted with stated amount of de-ionized, distilled water as mentioned on the label.

(a) Add accurately stated amount of distilled water to the lyophilized LYOPLASTIN<sup>®</sup> reagent. (b) Gently mix to dissolve. (c) Keep for 10 minutes and mix again gently ensuring complete resuspension of the lyophilized reagent. Avoid froth formation. (d) Thorough mixing should be ensured before withdrawing material every time for test purposes.

### SAMPLE COLLECTION AND PREPARATION OF PPP

Though no special preparation of the patient is required prior to sample collection by approved techniques, It is preferable that patients are not heavily exercised before blood collection. Fasting or only light non-fatty meals prior to blood collection provide samples with a desirable lower opacity.

Withdraw blood without undue venous stasis or 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 anticoagulated tubes, after detaching needle from the syringe. Do not delay mixing blood with anticoagulant. Avoid foam formation during mixing.

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. For occasional patients with haematocrit less than 20% or greater than 55%, this ratio must be readjusted to ensure valid results. Centrifuge immediately for 15 minutes at 1500 g on a laboratory centrifuge and transfer the plasma into a clean test tube. It should be ensured that the plasma is free from platelets (PPP). Cap the test tubes to prevent deterioration of samples. **Plasma must be tested preferably immediately.** However if the specimen are held at 22-24°C, then they may be tested within 2 hours and if the specimen is held at 2-4°C then they may be tested within 3 hours.

### TEST PROCEDURE

#### Manual Method

1. Aspirate from the reagent vial enough reagent for immediate testing requirements in a thoroughly clean and dry test tube. (Plastic test tubes are preferred).
2. Bring the reagent to room temperature before prewarming at 37°C for testing purpose.
3. Recap the reagent vial and replace immediately to 2-8°C.
4. To a 12 x 75 mm tube add 0.1 ml of plasma (PPP) and place the tube in a waterbath for 3 to 5 minutes at 37°C.
5. To the tube **forcibly** add 0.2 ml of LYOPLASTIN® reagent (prewarmed at 37°C for atleast 3 minutes) and simultaneously start a stopwatch. Shake the tube gently to mix contents.
6. Gently tilt the tube back and forth and stop the stopwatch **as soon as the first fibrin strand is visible and the gel/clot formation begins.** Record the time in 'seconds'.
7. Repeat steps 4-6 for a duplicate test on the same sample.
8. Find the average of the duplicate test values. This is the Prothrombin Time (PT).

**If a coagulation instrument is being used to perform the tests, the instrument manufacturers instructions must be strictly adhered to.**

### CALCULATION OF RESULTS

#### Manual Method

The results may be reported directly in terms of the mean of the double determination of PT of the test plasma in 'seconds'.

Or as a ratio 'R': **R = Mean of the patient plasma PT in seconds**

**MNPT for the reagent**

Or as International Normalized Ratio (INR), **INR = (R)<sup>ISI</sup>**, where ISI = International Sensitivity Index of the reagent (Refer reagent vial label).

It is recommended by the WHO that MNPT should be established for each lot of PT reagents by each laboratory, since PT results are dependent on the combination of reagent lot, instrument and technique followed at each laboratory. Usually plasma from atleast 20 normal healthy individuals should be used to establish the MNPT. The average of such PT results in seconds = MNPT.

### EXPECTED VALUES

Normal values using LYOPLASTIN® are between 11-15 seconds. Between manual and Turbo densitometric instrument results a variation of 1-2 seconds may be expected. For photo optical instruments, it is recommended that each laboratory must establish their own normal range. It is mandatory that each laboratory must establish its own MNPT for each lot of LYOPLASTIN®.

**Oral Anticoagulant Therapeutic range: INR = 2.0 - 3.5**

### REMARKS

1. It is recommended that controls with known factor activity should be run simultaneously with each test series to validate test run.
2. Incorrect mixture of blood and tri-sodium citrate, insufficient prewarming of plasma and reagent, contaminated reagents, glassware etc. are potential source of errors.
3. Oxalated plasma may induce prolonged clotting times.
4. Since the PT test functions correctly only at 37±0.5°C, temperature of all equipment must be calibrated daily.
5. 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.
6. Turbid, icteric, lipemic or grossly hemolysed samples may generate erroneous PT results.
7. Glasswares and cuvettes used in the test must be scrupulously clean and free from even traces of acids / alkalies or detergents.
8. Plasma samples held at 4-8°C may undergo 'cold activation' leading to a marked shortening of the PT.

9. The PT may be shortened during acute inflammatory conditions which are accompanied by increase in Fibrinogen levels and also by agents such as antihistamines, butabarbital, phenobarbital, caffeine, oral contraceptives and vitamin K. The PT may be prolonged by corticosteroids, EDTA, oral contraceptives, asparaginase, clofibrate, erythromycin, ethanol, tetracycline, aspirin and anticoagulants such as heparin and warfarin.
10. It is important that each laboratory express the results in terms of INR for patients on oral anticoagulant therapy for the clinician to adjust the dosage based on INR.
11. Since the test uses platelet poor plasma, each laboratory must calibrate the necessary force and time required during centrifugation to yield the PPP. Contamination of plasma with excess platelets could falsely elevate levels of some of the factors.
12. Homogenisation of LYOPLASTIN® reagent suspension before use is important to achieve accurate and consistent results.

### PERFORMANCE CHARACTERISTICS

#### Precision

The Precision of Prothrombin time determination is highly dependent on the method used. Precision studies were performed on Hemostar-XF coagulometer by assaying normal and abnormal control plasmas with LYOPLASTIN®. One normal control plasma and one abnormal control plasma in replicates of 10 were used to determine inter assay and intra-assay precision of the clotting times (seconds).

	Inter-assay precision			Intra-assay precision		
	Mean	SD	CV (%)	Mean	SD	CV (%)
Normal control plasma	14.6	0.45	3.08	14.1	0.26	1.84
Abnormal control plasma	33.8	2.22	6.56	32.1	0.62	1.93

#### Factor Sensitivity

LYOPLASTIN® is useful for measuring the deficiencies of factors of the extrinsic pathway. The factor sensitivity of LYOPLASTIN® was performed on Hemostar-XF (coagulometer based on turbodensitometric principle of clot detection) by diluting pool normal plasma with factor deficient plasmas in the range corresponding to 3.12 - 100 % activities.

Activity of Factor (%)	Clotting time with LYOPLASTIN® with factor deficient plasmas (seconds)			
	Factor VII	Factor X	Factor II	Factor V
100	23.6	38.6	27.5	33.5
50	28.7	50.8	37.9	40.5
25	35.2	70	53.7	47.4
12.5	44.8	90.8	74.6	58.2
6.25	56.1	125.9	104.4	66.3
3.12	69.9	150.6	160.8	78.9

The above values should only be used as guidelines. Each laboratory should establish sensitivity to individual factors using instruments, reagents and techniques used in their laboratory.

#### WARRANTY

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

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