Diagnostic Reagents Limited

UK CA

Freeze Dried Rabbit Brain Thromboplastin

Batch Number: DT
Catalogue Number: FRBT010 (5 mL)

For *in vitro* diagnostic use only.

Intended Use
Diagen Freeze Dried Rabbit Brain Thromboplastin is suitable for use in the One-stage Prothrombin Time (PT) and for the control of oral anticoagulant therapy.

Summary and Principle
The One-stage Prothrombin Time Test (PT) has become the basic coagulation screening test for the diagnosis of congenital and acquired deficiencies of Factors II, V, VII and X[1, 2]. Oral anticoagulant drugs inhibit hepatic synthesis of the vitamin K dependent clotting Factors II, VII, IX and X and thus the PT has become the method of choice to monitor the effects of Oral Anticoagulant Therapy (OAT) [3, 4].

In conjunction with the appropriate factor deficient substrate plasma, the PT may be modified to quantify specific clotting factor concentrations in plasma samples.

In the presence of Calcium ions, tissue thromboplastin initiates the extrinsic coagulation pathway by the direct activation of factor VII to VIIa. This culminates in the conversion of soluble Fibrinogen to insoluble Fibrin by the direct action of Thrombin. Reduction in the concentration of clotting factors of the extrinsic or common pathways will result in the prolongation of the PT, the degree of which is proportional to the level of concentration reduction. For OAT, the degree of prolongation is defined by the INR (International Normalized Ratio) and forms the basis of therapeutic anticoagulant control.

Reagent
Diagen Freeze Dried Rabbit Brain Thromboplastin 6 vials
A lyophilised saline extract of rabbit brain thromboplastin. For reconstitution, remove screw cap and bung then add 5.0 mL of distilled water to the contents of the vial and mix vigorously until the contents are dissolved.

Warnings and Precautions
Diagen Freeze Dried Rabbit Brain Thromboplastin contains components sourced from animal origin, passed fit for human consumption. Reagents containing animal products should be treated as potentially infectious. All wastes containing biological material should be correctly labeled and stored separately from other wastes. Waste materials should be disposed of according to prescribed international, national and local regulations. Please refer to the SDS Sheet (provided on request) for handling and safety procedures.

Collection of Blood Samples
Blood (9 parts) is collected into 1 part of 3.2% trisodium citrate and the plasma obtained by centrifugation at 2500 g for 15 minutes. The plasma should be stored in stoppered tubes. The use of 3.2% citrate containing 5% HEPES buffer improves the stability of both fresh and deep-frozen plasma.

Procedure
Materials Provided
Materials needed for Prothrombin time test are detailed below:
Cat. No.
FRBT010 - Freeze Dried Rabbit Brain Thromboplastin (6 x 5 mL)
Materials and equipment required, but not provided:
1. General routine laboratory coagulation equipment.
2. Reaction cups or test tubes (12 x 75 mm).
3. Pipettes delivering between 100 µL and 5.0 mL.
4. Distilled water.
5. 25 mM Calcium chloride.

6. Diagen Control plasmas: IQCN130 - Normal.
IQCM140 - Abnormal 1 (Mild).
IQCS150 - Abnormal 2 (Severe).

Manual Technique
1. 100 µL of Freeze-Dried Rabbit Brain Thromboplastin is placed in a clotting tube within a water bath at 37°C.
2. Incubate the Thromboplastin for 1 to 2 min to reach 37°C.
3. 100 µL of plasma is then added and incubated for exactly 1 minute.
4. 100 µL of 25 mM Calcium chloride (pre-warmed to 37°C) is then added and the stopwatch started.
5. The tube is gently tilted at regular intervals (returning to the water bath between tilting) and the time for the formation of a clot recorded. This is known as the Prothrombin Time (PT).

Notes:
1. Tubes should be new and scrupulously clean.
2. Water bath temperature should be 37°C.
3. Diagen Normal and Abnormal Internal Quality Control (IQC) plasmas can be used for day-to-day QC.
4) For photo-optical and mechanical instruments, please contact calibration@diagen.co.uk for further information.

Quality Control
All laboratories should have in place a quality control system that uses normal and abnormal controls to evaluate reagent, instrument and user performance. Both normal and abnormal controls should be used prior to performing a test series to validate the patient results. We recommend Diagen IQC plasmas for this purpose, as these have been specifically manufactured for our reagent. If the controls do not perform within their reference ranges, a review of the instrument or test system is recommended.

Control of Anticoagulant Therapy and International Calibration of Thromboplastins
Because of differing sensitivities of the methods used to control anticoagulant therapy, there is difficulty in comparing the level of anticoagulation at different centres. An approach to this problem was made by Biggs and Denson in 1967[5], who showed that it is possible to calibrate thromboplastin preparations in terms of their sensitivity to the anticoagulant defect, and to compare the sensitivity of any preparation against a selected reference material using the clotting time ratio method. The calibration is now performed by testing a number of samples from patients on stabilised anticoagulant therapy, together with normal plasma samples. The log clotting times for the test preparation are then plotted against those for the reference preparation and the best line obtained by orthogonal regression analysis. The slope of this line is termed the International Sensitivity Index (ISI), and using this slope, any clotting time ratio can be converted to the equivalent clotting time ratio for the Primary International Reference Preparation. The latter is termed the International Normalised Ratio (INR) and is the ratio that would have been obtained had the primary reference preparation been used for the patient's sample. A reference material coded 67/40 was prepared in 1967 and this was established by W.H.O. in 1976 as the first International Reference Preparation of thromboplastin.

Three secondary reference preparations of rabbit brain, ox brain and human brain were calibrated against 67/40 under the auspices of the Community Bureau of Reference of the E.E.C., W.H.O., I.C.S.H. and I.C.T.H [6, 7]. Further reference preparations of rabbit brain thromboplastin have since been made. **Diagen** Freeze Dried Rabbit Brain Thromboplastin has been calibrated against a secondary reference preparation which has in turn been calibrated against the IRP RBT/90 or Community Bureau of reference preparation CRM149S.

Automated Methods
The manufacturers' protocol should be followed. It is now well established that coagulometers may alter the ISI from the value obtained manually, and for this reason, each batch of reagent has a stated ISI for the **manual method only**.

If you wish to use the reagent on a specific instrument, please contact calibration@diagen.co.uk for further information.

Calculation of Results
The recommended method is to report the ratio calculated from the patients clotting time divided by the mean normal prothrombin time or MNPT (geometric mean of greater than 20 fresh donor samples) and then to convert this to an International Normalised Ratio (INR).

$$INR = PT\ ratio^{ISI}$$

e.g., for a PT ratio of 2.0 and an ISI of 1.38.

$$INR = 2.0^{1.38} = 2.6$$

The table (Figure 1) on page 4 shows INRs calculated from an ISI of 1.32 and an MNPT of 14.8 seconds.

Interpretation

Deficiency of factors II, V, VII, X or fibrinogen should result in a prolonged clotting time, thus increasing the PT ratio and raising the INR. During oral anticoagulant therapy, the dose is adjusted to keep the patient in the desired therapeutic range.

Therapeutic range: Based on long term experience, the therapeutic range for patients receiving oral anticoagulant treatment is INR 2.0 – 4.5. However, the degree of anticoagulation is determined by the reason for anticoagulant therapy and the patient's general condition. This decision should be made only by the clinician treating the patient.

Performance Characteristics

Replicate testing on 20 reagent vials tested on day 1 with abnormal reference plasma gave:

Manually SD= 0.247, CV = 0.748%

Replicate testing on 5 reagent vials tested on days 1-5 with abnormal reference plasma gave:

Manually SD= 0.427, CV = 1.292%

Batch Details

Lot No. **DT**

Expiry: **-**

	ISI	MNPT
Manual.....	**-**	**-**

Limitations

PT values will differ between laboratories due to the many variables that can affect clotting times. All laboratories should therefore establish their own quality control system. The use of icteric, lipemic, or haemolysed samples should be avoided as this may cause possible interference, especially when using photo-optical instruments. If the patient is on therapeutic drugs, in addition to oral anticoagulant therapy, it may influence interpretation of test results. By obtaining accurate patient history and noting specific drug therapies the potential impact on laboratory test results can be better understood. The presence of heparin or anti-phospholipid antibodies must always be considered in a sample where an abnormal result is obtained.

Storage and stability

The unopened vials are best stored deep frozen but may be stored for up to 3 years at 2 - 8°C without deterioration. Once reconstituted the contents of the vial are then stable for up to 5 days when held at 2 - 8°C.

Packaging

6 x 5.0 mL.

References

1) Quick A.J., The Prothrombin Time in Hemophilia and in Obstructive Jaundice. J. Biol. Chem:109,73-74;1935.
2) Biggs R. ed, Human Blood Coagulation Hemostasis and Thrombosis Second Ed. Blackwell Scientific Publications, London 1976.
3) Peterson C.E., Kwaan H.C., Current Concepts of Warfarin Therapy, Arch Intern. Med. 146:581-584, 1986.
4) Loeliger E.A:ICEH/ICTH Recommendations for Reporting Prothrombin Time in Oral Anticoagulant Control, Throm. Haemost. 53:155-156, 1985.
5) Biggs, R. and Denson K.W.E. Standardisation of the one-stage prothrombin time for the control of anticoagulant therapy. Brit Med J. 1967; 1: 84.
6) W.H.O. Expert Committee on Biological Standardisation. 33rd. Report. W.H.O. Tech. Rep. Ser. 1983.

7) Loeliger, E.A., van den Besselaar, A.M.H.P., Hermans, J. and van der Velde, E.A. The Certification of Three Reference Materials for Thromboplastins. BCR Information. Commission of the European Communities. 1984.

Clotting Time (Secs)	**INR**	Clotting Time (Secs)	**INR**	Clotting Time (Secs)	**INR**
14.4	**1.00**	27.0	**2.34**	38.5	**3.77**
15.0	**1.06**	27.5	**2.40**	39.0	**3.84**
16.0	**1.15**	28.0	**2.45**	39.5	**3.90**
17.0	**1.25**	28.5	**2.51**	40.0	**3.97**
17.5	**1.30**	29.0	**2.57**	40.5	**4.04**
18.0	**1.35**	29.5	**2.63**	41.0	**4.11**
18.5	**1.40**	30.0	**2.69**	41.5	**4.17**
19.0	**1.45**	30.5	**2.75**	42.0	**4.24**
19.5	**1.51**	31.0	**2.82**	42.5	**4.31**
20.0	**1.56**	31.5	**2.88**	43.0	**4.38**
20.5	**1.61**	32.0	**2.94**	43.5	**4.45**
21.0	**1.66**	32.5	**3.00**	44.0	**4.52**
21.5	**1.72**	33.0	**3.06**	44.5	**4.59**
22.0	**1.77**	33.5	**3.13**	45.0	**4.66**
22.5	**1.83**	34.0	**3.19**	45.5	**4.73**
23.0	**1.88**	34.5	**3.25**	46.0	**4.80**
23.5	**1.94**	35.0	**3.32**	46.5	**4.87**
24.0	**1.99**	35.5	**3.38**	47.0	**4.94**
24.5	**2.05**	36.0	**3.45**	47.5	**5.01**
25.0	**2.11**	36.5	**3.51**	48.5	**5.15**
25.5	**2.16**	37.0	**3.58**	50.0	**5.37**
26.0	**2.22**	37.5	**3.64**	51.0	**5.51**
26.5	**2.28**	38.0	**3.71**	54.0	**5.96**

Figure 1. Table showing INR values for clotting times calculated using a Thromboplastin with an ISI of 1.35 and MNPT of 14.4 seconds.