Diagnostic Reagents Limited

UK CA

Bovine Thrombin

Catalogue Number: **BTUA290 (20 units)**
BTUB291 (50 units)
BTUC292 (200 units)
BTUD293 (1000 units)

For *in vitro* diagnostic use only.

Intended Use
Diagen Bovine Thrombin has multiple applications including The Thrombin Clotting Time (TCT), the quantitative determination of Fibrinogen levels in citrated human plasma and the in the assay of Antithrombin.

Summary and Principle
Thrombin is the enzyme and penultimate protein in the clotting sequence; it acts upon soluble Fibrinogen converting it to insoluble Fibrin. Diagen Bovine Thrombin can be a used in the laboratory evaluation of Fibrinogen disorders, including Hypofibrinogenemia and Dysfibrinogenemia.

Plasma Fibrinogen concentration can be determined by the Clauss method [(1)]. The principle being that, varying levels of Fibrinogen concentration give a linear clotting time relationship (usually logarithmic transformation) with a standard concentration of Thrombin.

Diagen Bovine Thrombin can also be used for measuring the concentration of Antithrombin in human plasma [(2)].

Reagent
Bovine Thrombin **6 or 10 vials**
A lyophilised preparation of Bovine Thrombin prepared in 20, 50, 200 and 1000 units mL^{-1} concentrations. For reconstitution, remove the cap and rubber stopper and then add 1.0 mL of distilled water. Swirl gently and let stand undisturbed for 10 - 15 minutes before use or further dilution.

Warnings and precautions
Please refer to Diagen Bovine Thrombin SDS for further safety and handling information. Reagents containing animal products should be treated as potentially infectious. All wastes containing biological material should be properly labeled and stored separately from other waste. Waste materials should be disposed of according to prescribed international, national and local regulations. **Please note Diagen Bovine Thrombin is not for topical use.**

Storage and stability
The unopened freeze dried 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 stable for up to 24 hours when held at 2 - 8°C. The product may be deep frozen at -20°C and thawed once.

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
The materials provided are detailed below. Thrombin may be diluted for use.
Cat. No.
BTUA290 – 20 Units Bovine Thrombin (10 x 1.0 mL).
BTUB291 – 50 Units Bovine Thrombin (10 x 1.0 mL).
BTUC292 – 200 Units Bovine Thrombin (6 x 1.0 mL).
BTUD293 – 1000 Units Bovine Thrombin (6 x 1.0 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. Pipette delivering 100 µL, 200 µL and 1 mL.
4. Imidazole buffer (IMBX600).
5. Distilled water.

Technique
Diagen Bovine Thrombin has multiple applications including; Clauss method of Fibrinogen estimation and the Thrombin Time test.
The TCT may be performed as follows:
1. Dilute the Thrombin in buffer to give a working solution of 5-10 units mL^{-1}; this should give a clotting time of between 13 – 15 secs with normal reference plasma.
2. Add 200 µL of patient plasma to a test tube and warm to 37°C.
3. Add 100 µL of Bovine Thrombin and start the stopwatch.
4. The subsequent clotting time, is the Thrombin Clotting Time. It is recommended that the test is performed in duplicate.
For photo-optical and mechanical instruments, always follow the manufacturer's instructions.

Interpretation
The Thrombin Clotting Time is prolonged in the presence of Heparin or heparin like inhibitors, Hypofibrinogenaemia, Dysfibrinogenaemia, Dysprotienaemia and in the presence of Fibrin Degradation Products.

Expected results
The normal value for the TCT will vary with instrumentation and techniques used, each laboratory should determine their own working Thrombin concentrations and reference range.

Quality Control
All laboratories should have in place a quality control system that uses plasma controls to validate the assay, thus evaluating instrument, reagent and user performance. Diagen Normal and Abnormal Reference Control Plasmas have calibrated values for both fibrinogen concentration and TCT that may be used for this purpose.

Limitations
The inhibitory effect of therapeutic levels of Heparin may cause incoagulability in the TCT. Neutralising the Heparin in the plasma with Protamine sulphate or Polybrene should correct the TCT.

Packaging
10 x 1.0 mL (20 and 50 units) or 6 x 1.0 mL (200 and 1000 units).

References
1. Clauss A. Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens, Acta Haemat. 1957; 17:237.
2. Biggs, R., Denson, K.W.E., Akman, N., Borrett, R. and Haddon, M. Antithrombin III, Antifactor Xa and Heparin. Brit. J. Haemat. 19, 3 (1970).

Key guide to symbols

REF Manufacturers catalogue number.
Consult instructions for use.
LOT Manufacturers batch number.
Recon. Requires reconstitution.
IVD For *in vitro* diagnostic use only.
Product expiry date.
Biological risks.
Store at 4°C or below. Best stored deep frozen.

Manufacturer.

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