

Bio/Data Corporation

UPTT™ REAGENT

Non-activated Partial
Thromboplastin Time Reagent

Contact Activation Screening Reagent
For The Study of the Activation
of the Intrinsic Coagulation System

PRODUCT DESCRIPTION

UPTT is a lyophilized preparation of rabbit brain cephalin. The standardized rabbit brain phospholipids concentration is $\leq 0.1\%$. The UPTT reagent supplies the phospholipids required for plasma coagulation by way of the intrinsic hemostatic pathway.

INTENDED USE

RESEARCH USE ONLY

UPTT is for use in performing a non-activated Partial Thromboplastin Time Test [PTT]. In hemocompatibility studies, the PTT measures contact activation of the intrinsic coagulation system. The PTT is a widely used test for measuring the activation of factors XI and XII.^{1,2}

PRINCIPLE

Contact activation occurs in the absence of Ca^{++} following whole blood exposure to a foreign material.¹³ The PTT is a simple and reliable measurement of the activation of the intrinsic coagulation pathway.⁵ The PTT is a modification of the plasma recalcification time test. The UPTT introduces a platelet substitute, which eliminates test variability due to availability of platelet phospholipids.³

PRECAUTIONS

UPTT is for RESEARCH USE ONLY and is NOT FOR USE IN HUMAN DIAGNOSIS OR TREATMENT.

MATERIALS PROVIDED

UPTT Reagent 15 x 10mL
UPTT Reagent 20 x 3.0mL

Store at $2 - 8^{\circ}\text{C}$ prior to reconstitution.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Purified water, pH 5.3 – 7.2 [distilled, deionized or reagent grade]
2. Pipettes [10.0, 3.0, and 0.1mL volume capabilities]
3. Calcium Chloride 0.025 M [see PRODUCT AVAILABILITY]
4. Citrated control plasmas [see PRODUCT AVAILABILITY]

INSTRUMENTATION

Non-activated Partial Thromboplastin Time endpoints may be detected by manual methods or by most semi-automated or automated coagulation analyzers. The recommended test methods are the same as those used for the activated Partial Thromboplastin Time (aPTT). Follow the manufacturer's instructions for the analyzer in use.

COLLECTION AND PREPARATION OF TEST PLASMA

Test plasma for the UPTT must be platelet poor plasma prepared from anticoagulated whole blood. The whole blood specimen must not be exposed to glass surfaces during collection, processing or testing.

1. Centrifuge blood at $2500 \times g$ for 15 minutes
2. Remove plasma from cells without disturbing the buffy coat
3. Transfer the plasma to a plastic test tube
4. If testing is delayed, store the plasma at $2 - 8^{\circ}\text{C}$ for a maximum of two hours.

Note: A 9 to 1 blood to anticoagulant ratio should be adjusted based on the hematocrit. UPTT should not be performed on visibly hemolyzed or lipemic plasma.

RECONSTITUTION

Warm vial to room temperature prior to reconstitution.

1. Tap vial to dislodge material adhering to stopper
2. Remove the aluminum seal
3. Remove the stopper and reconstitute the vial contents with the volume of purified water specified on the label
4. Replace the stopper and invert the vial to thoroughly mix the contents. Let stand for not less than 15 minutes prior to use to assure complete rehydration of the contents.

Once reconstituted, UPTT is stable for seven days when stored in the tightly sealed original container at $2 - 8^{\circ}\text{C}$.

TEST PROCEDURE

NOTE: THIS TEST PROCEDURE IS FOR MANUAL METHODOLOGY. FOR USE WITH AUTOMATED OR SEMI-AUTOMATED ANALYZERS, FOLLOW THE INSTRUCTIONS IN THE ANALYZER'S OPERATIONS MANUAL.

Prepare the test plasma as specified by your protocol. The amount of time that the material being evaluated is in contact with the test plasma must be precisely controlled.

NOTE: FOR BEST RESULTS, SUSPENSION OF THE UPTT REAGENT SHOULD BE MAINTAINED EITHER BY MAGNETIC STIRRING OR GENTLE INVERSION IMMEDIATELY PRIOR TO USE

1. Pipette 0.1mL test or control plasma into a test cuvette and incubate the plasma for 2 minutes at 37°C .
2. Pre-incubate 0.025 M Calcium Chloride at 37°C .
3. Pipette 0.1mL UPTT Reagent into the test cuvette containing the plasma.
4. Incubate the plasma/reagent mixture for 2 to 5 minutes at 37°C or as specified by your protocol.
5. Add 0.1mL of the pre-incubated calcium chloride, simultaneously starting a timer.
6. Record the clotting time

QUALITY CONTROL

The precision and accuracy of UPTT test results may be influenced by a number of factors. Significant protocol and inter-laboratory differences arise from the number of coagulation systems used to measure the clotting end point.^{7,14} Intra-laboratory variables that may affect test results include pH of the purified water used for reagent reconstitution, pipetting technique, incubation time and temperature, reagent contamination, or change in reagent lot number.⁸ Periodic quality control tests, performed on a regular basis, will help to identify any variations that may occur and lead to erroneous test results.

Each laboratory should determine acceptance limits for each lot of UPTT Reagent and control plasmas used by performing replicate studies in accordance with established laboratory procedures. Comparison to Activated Partial Thromboplastin Time (APTT) results run in the same fashion may be useful.

RESULTS

The UPTT result is reported as the clotting time endpoint in seconds. In addition to the clotting time, a predetermined reference range should be reported because UPTT ranges may vary substantially from laboratory to laboratory.⁸⁻¹⁰

EXPECTED VALUES

UPTT results, when tested with normal control plasma on a photo-optical analyzer, will be greater than 60 seconds. Contact activation will shorten this time. Because of the operational variability among analyzers and protocols, each laboratory must establish a reference range for the UPTT. The experimental protocol, sample preparation and endpoint detection method may influence test results.

LIMITATIONS

1. Grossly hemolyzed or lipemic plasma may produce erroneous test results.
2. Freezing plasma samples may cause erroneous test results¹³
3. Incorrect ratio of blood to anticoagulant may cause spurious test results.¹⁰ The quantity of anticoagulant added to blood must be proportionally decreased in specimens with hematocrit values above 53%, and increased for those with hematocrit values below 25%.¹⁰
4. The use of analyzers with a mechanical or combined optical-mechanical detection system may contribute to contact activation and result in undetected or erroneous test results.

PERFORMANCE CHARACTERISTICS

UPTT has been tested in photo-optical end point detection systems. Its precision and sensitivity are sufficient to provide a reliable method for evaluating contact activation of the intrinsic coagulation system.¹¹

REFERENCES

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2. Biological Evaluation of Medical Devices, part 4: Selection of tests for interaction with blood. (Committee Draft ISO 10993-4). Washington D.C. Feb. 1999
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4. Hougie C: The Biochemistry Of Blood Coagulation. In Triplett DA: Laboratory Evaluation of Coagulation, pg 2 American Society of Clinical Pathologists Press, Chicago, 1982.
5. Owen CA, Bowie EJW, Thomson JH: The Diagnosis Of Bleeding Disorders, pg 110, Little Brown and Company, Boston, 1975.
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8. Westgard JO, Barry PL, Hunt MR, Groth T: A Multi-Rule Shewhart Chart For Quality Control In Clinical Chemistry Clin Chem. 27(3), 493-501, 1981.
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10. Harker LA: Hemostasis Manual. F.A. Davis Company, pg 62, Philadelphia, 1974.

11. Ratnoff OD, Forbes CD eds: Disorders of Hemostasis. W.B. Saunders Co. 2nd ed, Philadelphia, 1991.
12. Lindout T. Biocompatibility of Extracorporeal Blood Treatment: Selection of Hemostatic Parameters. Nephrol Dial Transplant 1994; 9 Suppl 2: 83-9
13. Harmening, DM. ed. Clinical Hematology and Fundamentals of Hemostasis. 3rd ed. pg 542. F. A. Davis and Company, Philadelphia. 1997.
14. Poller L. Standardization of the aPTT Tests. Current Status, Scand J Haematol. 25[Suppl 37]:49, 1980.

PRODUCT AVAILABILITY

PRODUCT	NET CONTENTS	CATALOG NUMBER
UPTT™ Reagent	15 x 10.0mL	105905
UPTT™ Reagent	20 x 3.0mL	105997

ALSO AVAILABLE

Calcium Chloride Solution 0.025M	473mL	100989
Coagulation Control Plasmas		
Citrex® I (Normal)	20 x 1.0mL	101166
Citrex® II (Abnormal)	20 x 1.0mL	101170
Citrex® III (Abnormal)	20 x 1.0mL	101174

For a full product listing of coagulation and platelet aggregation products, contact Customer Service or visit our web site.

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