

Catalogue # 1479 (ROW)

MiniQuant[®] D-dimer

Quantitative Latex Agglutination Test
For Fibrin D-dimer
Reagents for 2 x 50 tests
For Use with MiniQuant[®]-1 Instrument
For *in vitro* diagnostic use

Lot #: XXXXXX/XX

Expiry date: XXXX XX XX

FOR INFORMATION USE ONLY
Not to be used for performing the assay.
Refer to the insert accompanying kit

I. INTENDED USE

Biopool[®] MiniQuant[®] D-dimer is an immunoturbidimetric assay used for the quantitative determination of the fibrin degradation products that contain D-dimer in human citrated plasma. The MiniQuant[®] D-dimer reagents are intended to be used only on the Biopool[®] MiniQuant[®]-1 instrument.

II. SUMMARY

D-dimer containing moieties are formed by plasmin degradation of factor XIIIa cross-linked fibrin. Elevated levels of D-dimer are found in clinical conditions such as deep vein thrombosis (DVT), pulmonary embolism (PE) and disseminated intravascular coagulation (DIC).¹⁻³ Laboratory measurements of fibrin degradation products, including D-dimer, have significance in screening for these conditions.

III. PRINCIPLE

Biopool[®] MiniQuant[®] D-dimer is a turbidimetric assay that utilizes antibody coated latex particles. In the presence of D-dimer, the particles aggregate and turbidity increases. The increase in scattered light is proportional to the amount of D-dimer in the sample. The latex particles are coated with a monoclonal antibody that reacts with fibrin D-dimer or fragment D of fibrin. The antibody has no cross reactivity with fibrinogen.⁴ This allows for the determination of D-dimer in human plasma.

IV. REAGENTS

A. Reagent Description

The reagents are lot-specific. Lots are not interchangeable.

1. Latex Reagent

2 x 2.5 ml of 0.30% latex reagent in HEPES buffer pH 8.5, with 0.2 g/L sodium azide, protein stabilizers and detergents.

2. Reaction Buffer

2 x 2.5 ml of HEPES buffer pH 7.0, containing 0.2 g/L sodium azide and detergents.

3. Saline Solution

2 x 8 ml of buffered saline pH 7.3, containing 0.2 g/L sodium azide. Used for reconstitution of lyophilized reagents and sample dilutions.

4. D-dimer Standard 0 µg/L

2 vials of lyophilized plasma immunodepleted for D-dimer, used as 0 µg/L standard.

5. D-dimer Standard 3200 µg/L

2 vials of lyophilized human plasma enriched with D-dimer at a level of approximately 3200 µg/L. See section IV. B. point 4 for lot-specific assayed value.

6. D-dimer Low Control

2 vials of lyophilized human plasma enriched with D-dimer at a level of approximately 300 µg/L. See section IV. B. point 5 for lot-specific assayed value.

7. D-dimer High Control

2 vials of lyophilized human plasma enriched with D-dimer at a level of approximately 2000 µg/L. See section IV. B. point 5 lot-specific assayed value.

B. Reagent Preparation

1. Latex Reagent

The latex may sediment during storage. Mix thoroughly before use.

2. Reaction Buffer

Ready for use.

3. Saline Solution

Ready for use.

4. D-dimer Standards 0 and 3200 µg/L

Reconstitute by adding 1.0 ml of Saline Solution to each vial. Gently agitate for 5 minutes at room temperature, until content is fully dissolved.

Standard 3200 µg/L:

Lot #: XXXXXX/XX

Lot Specific D-dimer concentration: XXXX mg/L SD = XXX mg/L

Using the lot-specific D-dimer concentration for calibration of the MiniQuant[®] D-dimer kit

Make a serial dilution of the D-dimer Standard 3200 µg/L with the D-dimer Standard 0 µg/L using the lot-specific value of the standard as described in the table below. Program a standard curve on the MiniQuant[®]-1 instrument using these values. Follow the Assay procedure as described in section VIII. 3.

Conc (mg/L)	0 mg/L standard	Mix with
XXXX	0 µl	---
XXX	100 µl	100 µl XXX µg/L standard
XX	100 µl	100 µl XXX µg/L standard
X	100 µl	100 µl XXX µg/L standard
	100 µl	100 µl XXX µg/L standard
	100 µl	100 µl XXX µg/L standard

5. D-dimer Low and High Controls

Reconstitute by adding 1.0 ml of Saline Solution to each vial. Gently agitate for 5 min at room temperature, until content is fully dissolved.

Lot-specific values: Assigned Value Recommended Range

D-dimer Low Control

XXX mg/L

XXX – XXX mg/L

Lot #: XXXXXX/XX

D-dimer High Control

XXXX mg/L

XXXX – XXXX mg/L

Lot #: XXXXXX/XX

V. STORAGE AND STABILITY

All unopened and unreconstituted reagents are stable until the expiration date stated on the box and vial labels when stored at 2-8°C.

1. Latex Reagent

When opened, stable for 4 weeks at 2-8°C. To reduce prolonged storage at elevated temperatures, an aliquot of the latex suitable for daily work should be transferred to a smaller vial, e.g. a 2 ml screw capped polypropylene "cryo-type" vial. Place this vial in the incubator position and allow the latex to equilibrate at the working temperature of the instrument (37°C) for 15 minutes before use. Return to 2-8°C at end of working day. When handled in this way, the latex may be stored for 8 hours a day at 37°C during 10 working days without appreciable loss of reactivity.

2. Reaction Buffer

When opened, stable for 4 weeks at 2-8°C.

3. Saline Solution

When opened, stable for 4 weeks at 2-8°C.

4. D-dimer Standards 0 and 3200 µg/L

Stable for 10 hours at 20-25°C.

5. D-dimer Low and High Controls

Stable for 10 hours at 20-25°C or 1 week at 2-8°C.

A single freeze-thaw cycle involving 4 weeks of storage at -20°C does not affect the assay response.

VI. WARNINGS AND PRECAUTIONS

The Biopool® MiniQuant® D-dimer standards and controls are of human origin. Each donor unit of source plasma used in these products has been tested and found negative for Hepatitis B antigens, HIV I and II antibodies, Hepatitis C antibodies, syphilis antibodies and H.T.L.V. I/II antibodies by FDA approved methods. However no test can offer complete assurance that products derived from human blood will not transmit infectious disease. As with all materials of human origin, this product should be handled as a potentially infectious agent. All wastes containing biological material should be properly labelled and stored separately from other wastes. Dispose of all waste materials according to prescribed international, national and local regulations.

The Saline Solution, Reaction Buffer and Latex Reagent contain sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Materials discarded into a sink should be flushed with a large volume of water to prevent azide build-up.

The test should be used in conjunction with clinical observations and results of other laboratory tests.

VII. SPECIMEN COLLECTION

Nine volumes of blood are collected in one volume of 0.1 M trisodium citrate, followed by centrifugation at 3000 x g for 10 min. Citrate plasma samples may be stored at room temperature for 2 h, at 2-8°C for 8 h.

VIII. PROCEDURE

A. Material provided

- Latex Reagent
- Reaction Buffer
- Saline Solution
- D-dimer Standards, 0 and 3200 µg/L
- D-dimer High and Low Control

B. Materials required but not provided

- Biopool® MiniQuant®-1 instrument, Cat #1466
- MiniQuant® Cuvettes, Cat #1469
- Pipettes for 25, 50, 100 and 1000 µl
- Pipette tips
- Test tubes

1. Instrument

Follow Operators Manual for the installation, calibration, and operation of the MiniQuant®-1 instrument for performance of D-dimer assays.

2. Preparation of standard curve

Make a serial dilution of the D-dimer Standard 3200 µg/L with the D-dimer Standard 0 µg/L as described in the table below.

Conc (µg/L)	0 µg/L standard	Mix with
3200	0 µl	---
1600	100 µl	100 µl 3200 µg/L standard
800	100 µl	100 µl 1600 µg/L standard
400	100 µl	100 µl 800 µg/L standard
200	100 µl	100 µl 400 µg/L standard
100	100 µl	100 µl 200 µg/L standard

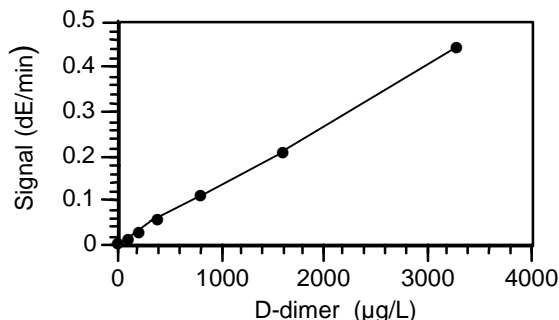
The dilutions are stable for 10 hours at room temperature when kept in a capped tube. Analyze duplicate samples of each standard concentration, calculate the mean value of each concentration and program a standard curve according to instructions accompanying the MiniQuant®-1 instrument.

Alternatively, use the lot-specific value for D-dimer Standard 3200 µg/L given in section IV. B. 4 Reagent Preparation to determine the exact D-dimer concentration in each standard dilution.

Users must construct a standard curve each time a new kit lot is used, or every 6 months, and when Control Plasma is assayed out of range. A typical standard curve is shown below.

Note:

- Do not use, for demonstration purpose only.
- The zero point is automatically entered by the instrument.
- The instrument uses a point-to-point calibration method.



3. Assay procedure

- Transfer latex reagent corresponding to daily consumption to a screw capped vial and place in reagent compartment of the MiniQuant®-1 instrument.
- Place cuvettes (duplicates for each sample to be analyzed) in incubation area of the instrument.
- Add 50 µl Reaction Buffer to the cuvettes.
- Transfer 25 µl plasma sample to each cuvette. Incubate 2-4 minutes.
- Transfer two cuvettes containing sample and buffer to the measuring positions.
- Initiate instrument reading by pressing the key sequences "Optic 1" and "Optic 2". Enter patient identification data if desired.
- Activate the instrument by again pressing the key sequences "Optic 1" and "Optic 2".
- Add 50 µl Latex Reagent to the cuvette in the first measuring position.
- Mix thoroughly by multiple aspirations of the reaction mixture for not more than 9 seconds. **NOTE:** Keep pipette tip below liquid surface to avoid the introduction of air bubbles.
- Repeat addition of 50 µl Latex Reagent for the cuvette in the second measuring position.

IX. RESULTS

The results are automatically calculated by the MiniQuant®-1 Instrument. The instrument can calculate, display, and print the D-dimer concentration in 4 different units; µg/L, mg/L, µg/ml, and ng/ml. The units are related as follows:

$$1000 \mu\text{g/L} = 1000 \text{ ng/ml} = 1 \text{ mg/L} = 1 \mu\text{g/ml}.$$

X. QUALITY CONTROL

It is recommended that the Control Plasmas D-dimer Low Control and D-dimer High Control are assayed at regular intervals in order to ensure consistent assay results. If the control plasma result deviates from the D-dimer concentration given in the lot-specific Instruction for Use, a new standard curve should be constructed.

XI. LIMITATIONS AND INTERFERENCES

No interference was found from bilirubin (<0.26 g/L), hemoglobin (<6.7 g/L) or triglycerides (<2.5 g/L). However, highly lipemic samples should be diluted in Saline Solution and re-assayed since elevated levels of triglycerides may cause depressed D-dimer results (approximately 80% recovery at 8 g/L triglyceride).

Presence of rheumatoid arthritis factor may result in false-positive results (influence not quantified).

XII. EXPECTED VALUES

In a study of 132 normal individuals, 95% of the values were below 250 µg/L. Elevated levels are found in patients with confirmed deep venous thrombosis (DVT), pulmonary embolism, DIC, and trauma.¹⁻³ D-dimer levels rise during pregnancy and high levels are associated with complications.⁵

The circulatory half-life of D-dimer is about 12 h. Elevated D-dimer levels can, therefore, persist for some time after the active process has ceased.

Each laboratory must determine reference intervals for their individual test populations.

XIII. PERFORMANCE CHARACTERISTICS

A. Correlation

When compared to another quantitative immunoturbidimetric method, the Biopool® MiniQuant® D-dimer assay correlated as follows:

$$y = 0.90x + 44.5, r^2 = 0.93, N = 222 \text{ samples}$$

B. Precision

Within run and run-to-run precision resulted in CV's of ≤ 10% when tested at ranges from 250 to 3200 µg/L D-dimer.

C. Reproducibility

To evaluate lot-to-lot reproducibility, 20 replicate samples of D-dimer Low Control and D-dimer High Control were assayed on a single MiniQuant®-1 instrument with 3 different lots of reagent. All CV's were ≤ 10%.

D. Accuracy/Recovery

Accuracy and recovery of the MiniQuant® D-dimer assay system was evaluated by preparing dilutions of D-dimer Standard 3200 µg/L to known levels. Four replicate determinations were made of each sample. Known D-dimer values in the range 75-2400 µg/L recovered at ±10% versus the expected value in all cases.

E. Sensitivity and Assay range

The lower limit of detection is 75 µg/L. The assay range is 75-3200 µg/L. Samples above 3200 µg/L should be diluted with Saline Solution and re-assayed. Results below 75 µg/L should be reported as ≤ 75 µg/L.

F. Specificity

The monoclonal antibody used in this device, MA-8D3, is specific for D-dimer by virtue of the screening method used for hybridoma selection.¹ A hybridoma, secreting antibodies with a 1000-fold greater affinity for purified D-dimer and fragment D of fibrin, than for native fibrinogen, was selected.⁴ Clinical studies have confirmed the ability of this antibody to discriminate between DVT cases and controls.^{2,3}

XIV. REFERENCES

1. Declerck, P., *et al.* Fibrinolytic response and fibrin fragment D-dimer levels in patients with deep vein thrombosis. *Thrombosis and Haemostasis* 58, 1024-1029, 1987.
2. Lindahl, T., *et al.* Clinical evaluation of a diagnostic strategy for deep venous thrombosis with exclusion by low plasma levels of fibrin degradation product D-dimer. *Scand. J. Clin. Lab. Invest.* 58, 307-316, 1998.
3. Hansson P.O., *et al.* Can laboratory testing improve screening strategies for deep vein thrombosis at an emergency unit? *J. Intern. Med.* 235, 143-151, 1994.
4. Holvoet, P., *et al.* Binding properties of monoclonal antibodies against human fragment D-dimer of cross-linked fibrin to human plasma clots in an in vivo model in rabbits. *Thrombosis and Haemostasis* 61, 307-313, 1989.
5. Ballegeer, V., *et al.* Fibrinolytic response to venous occlusion and fibrin fragment D-dimer levels in normal and complicated pregnancy. *Thrombosis and Haemostasis* 58, 1030-1032, 1987.

XV. KEY GUIDE TO SYMBOLS



Use by



Lot



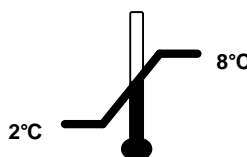
Catalogue number



Manufacturer



For *in vitro* diagnostic use



Store at 2-8°C



Consult accompanying documents



Biological risks

Recon.

Reconstitute with

Manufactured by:
Trinity Biotech plc,
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Ireland.
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Web: www.trinitybiotech.com

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