Monobind Inc.
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AccuLite
CLIA Microwells

D-Dimer Test System
Product Code: 12075-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of D-Dimer Concentration in Human Plasma and Serum by a Microplate Enzyme Immunoassay, Chemiluminescense

2.0 SUMMARY AND EXPLANATION OF THE TEST

D-Dimer is the term for the cross-linked dimer of fibrinogen degradation product (FDP) D. After fibrinogen is formed in a blood clot, it is broken down through a series of steps so that it can be cleared from the body. D-Dimer is the endpoint of this process which makes its elevation a useful marker for activation of the coagulation and fibrinolytic systems.[1]

D-Dimer concentration may be determined by a blood test to help diagnose thrombosis. Since its introduction in the 1990s, it has become an important test performed in patients with suspected thrombotic disorders. While a negative result practically rules out thrombosis, a positive result may indicate thrombosis but does not definitively rule out other potential causes.[1] Its main use, therefore, is to exclude thromboembolic disease where the probability is low. Additionally, the D-Dimer test can be used in the diagnosis of disseminated intravascular coagulation.[1]

In general circumstances, a D-Dimer value under 500 ng/ml fibrin equivalence units (FEU) excludes deep vein thrombosis (DVT), pulmonary embolism (PE) and other venous thromboembolism (VTE). However, baseline D-Dimer levels increase with age and during pregnancy so modified cut-off values should be implemented for these types of patients to minimize false-positive results.[2-3]

Monitoring D-Dimer levels has become increasingly important as a positive result is indicative of an increased mortality risk. Specifically, detecting high levels of D-Dimer in cancer and pulmonary infection patients is important. VTE is the second highest cause of death in patients with cancer while significant increases in D-Dimer have been linked to higher mortality in those suffering from lung diseases such as COVID-19.[4-6]

The D-Dimer AccuLite® Test System is a quantitative test designed to be sensitive across a wide range of D-Dimer values. The reagents utilize monoclonal mouse antibodies to create a sandwich complex via a simple, fast, and user-friendly protocol.

3.0 PRINCIPLE

Immunoenzymometric sequential assay (TYPE 4):

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen.

Upon mixing assay buffer and a serum containing the native antigen, reaction results between the native antigen and the coated antibody, forming an antibody-antigen complex. This interaction is illustrated below:

$$Ag + Ab_{(C)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{(C)}$$

$Ab_{(C)}$ = Coated Antibody (Excess Quantity)
Ag = Native Antigen (Variable Quantity)
$AgAb_{(c)}$ = Antigen-Antibody complex (Variable Quant.)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-coated-antibody complex on the surface of the wells.

$$AgAb_{(C)} + Ab_{(Enz)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} Ab_{(Enz)}AgAb_{(C)}$$

$AgAb_{(c)}$ = Antigen-Antibody complex (Variable Quant.)
$Ab_{(Enz)}$ = Enzyme-labeled Antibody (Excess Quant.)
$Ab_{(Enz)}AgAb_{(C)}$ = Sandwich complex (Variable Quant.)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

After another incubation period, the excess enzyme-labeled antibody is separated by washing. The remaining complex is then quantified by addition of substrate that reacts with bound enzyme. The amount of antigen is directly related to the amount of substrate converted by the enzyme.

4.0 REAGENTS

Materials Provided:

A. D-Dimer Calibrators – 1 ml/vial - Icons A-F
Six (6) vials of references for D-Dimer Antigen at levels of 0(**A**), 100(**B**), 400(**C**), 1500(**D**), 4000(**E**) and 10000(**F**) ng/ml FEU. Store at 2-8°C. A preservative has been added.

B. D-Dimer Control – 1 ml/vial – Icon M
One (1) vial of reference control for D-Dimer. Store at 2-8°C. A preservative has been added.

C. Assay Buffer – 12 ml/vial - Icon Ⓑ
One (1) vial containing buffer, dye, and preservatives. Store at 2-8°C.

D. D-Dimer Tracer Reagent —13 ml/vial - Icon Ⓔ
One (1) vial containing enzyme labeled affinity purified anti-D-Dimer monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

E. D-Dimer Light Reaction Wells – 96 wells – Icon
One 96-well white microplate coated with monoclonal anti-D-Dimer mouse IgG and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

F. Wash Solution Concentrate – 20 ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

G. Signal Reagent A – 7ml/vial - Icon C[A]
One (1) vial containing luminol in buffer. Store at 2-8°C.

H. Signal Reagent B – 7ml/vial - Icon C[B]
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

I. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are enough for a single 96-well microplate assay.

4.1 Required but not provided:

1. Pipette capable of delivering 0.025ml (25µl) and 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Luminometer
5. Test tube(s) for mixing signal reagents A&B.
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high levels for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.

2. **Working Signal Reagent Solution -** Store at 2 – 8°C.
   Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) eight well strips (A slight excess of solution is made). **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).

*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed. (Duplicate is recommended) **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of Assay Buffer to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 20 minutes at room temperature
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.**
8. Add 0.100 ml (100µl) of D-Dimer-Enzyme Reagent to all wells. **DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION**
9. Incubate 20 minutes at room temperature.
10. Wash the wells five (5) times by following steps 6 and 7 as above.
11. Add 0.100 ml (100µl) of working signal reagent to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells**
    DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION
12. Incubate at room temperature in the dark for five (5) minutes.
13. 10. Read the relative light units in each well with a chemiluminescence microplate reader for 0.5-1.0 seconds. **The results should be read within 30 minutes after adding signal.**

10.0 CALUCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of D-Dimer in unknown specimens.

1. Record the RLUs obtained from the printout of the microplate luminometer as outlined in Example 1.
2. Plot the light intensity for each duplicate serum referenceversus the corresponding D-Dimer concentration in ng/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of D-Dimer for an unknown, locate the average RLUs of the unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLUs (26982) of the unknown intersects the calibration curve at (1724 ng/ml) D-Dimer concentration (See Figure 1).

Note: Computer data reduction software designed designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained. Duplicates of the unknown may be averaged as indicated (see Figure 1).

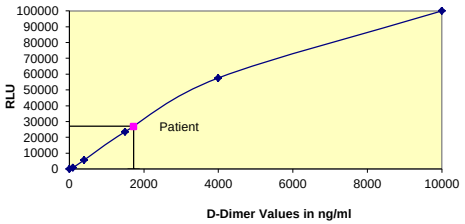
EXAMPLE 1

Sample I.D.	Well Number	RLU (A)	Mean RLU (B)	Value (ng/ml)
Cal A	A1	18	19	0
	B1	20		
Cal B	C1	966	961	100
	D1	956		
Cal C	E1	5684	5706	400
	F1	5728		
Cal D	G1	23627	23601	1500
	H1	23575		
Cal E	A2	57402	57610	4000
	B2	57817		
Cal F	C2	100359	100000	10000
	D2	99641		
Ctrl 1	E2	1744	1760	**164**

	F2	1775		
Ctrl 2	G2	53796	53174	**3625**
	H2	52553		
Patient	A3	26930	26982	**1724**
	B3	27033		

* The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the F calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Patient specimens with D-Dimer concentrations above 250ng/ml may be diluted with normal male serum (D-Dimer<1ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.

2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. **A D-Dimer value alone is not of diagnostic value** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures.

13.0 EXPECTED RANGES OF VALUES

The expected D-Dimer levels for exclusion of thrombosis in plasma samples were obtained from published literature.[2-3, 7] There is general consensus for the following data.

Table 1: Expected Plasma D-Dimer Levels

Patient Age	D-Dimer Level to Exclude Thrombosis
<50 years	<500 ng/ml FEU
>50 years	<Age x 10 ng/ml FEU

To obtain a reference range for serum samples, D-Dimer levels were measured by the D-Dimer AccuLite® Test System in apparently normal adults of different age groups. The values obtained are shown in Table 2.

Table 2: Serum D-Dimer Ranges

Patient Age (years)	N	Average (ng/ml FEU)	Highest (ng/ml FEU)	Lowest (ng/ml FEU)
<50	23	326	1244	137
50-59	19	486	1375	161
60-69	11	467	859	180
70-79	3	440	808	230
80+	3	878	1206	311

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The intra-assay precision of the D-Dimer AccuLite® CLIA test system was determined by measuring sixteen (16) replicates of three levels of patient control pools on the same assay run. The results are shown in Table 3.

Table 3: Intra-assay Precision

Sample	N	Mean (ng/ml)	σ	CV%
Control 1	16	145	6.3	4.3
Control 2	16	1811	75	4.1
Control 3	16	4124	154	3.7

The inter-assay precision (total precision) of the D-Dimer AccuLite® CLIA test system was determined by measuring three levels of patient control pools on three different kits throughout the course of two months. The results are given in Table 4.

Table 4: Inter-assay Precision

Sample	N	Mean (ng/ml)	σ	CV%
Control 1	24	151	9.1	6.0
Control 2	24	1940	112	5.8
Control 3	24	3800	245	6.5

14.2 Sensitivity

The D-Dimer AccuLite® CLIA test system has an analytical sensitivity of 2.78 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml calibrator and using the 95% confidence statistics (N=144) to calculate the minimum dose.

14.3 Accuracy

14.3.1 Linearity

The linearity of the D-Dimer AccuLite® CLIA Test System was tested by serially diluting several human plasma and serum samples containing high levels of D-Dimer (up to 11,000 ng/ml) with the "0 ng/ml" serum reference. The observed values were plotted against the expected values and the test system was determined to have excellent linearity up to 11,000 ng/ml with a slope of 0.977 and a correlation factor (R^2) of 0.998.

14.3.2 Recovery

Several human plasma and serum samples containing low levels of D-Dimer (100-700 ng/ml) were spiked with 100, 400, 1200, 4000, and 8000 ng/ml of D-Dimer and assayed on the D-Dimer AccuLite® CLIA Test System. The system demonstrated excellent recovery with all observed values falling within 15% of the expected values.

14.4 Specificity

The cross-reactivity of this method to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Chorionic Gonadotropin needed to produce the same light intensity.

Substance	Cross Reactivity	Concentration
D-Dimer	1.0000	----
Fibrogen	< 0.0001	4mg/ml
Plasminogen	< 0.0001	150ng/ml
Angiostatin	< 0.0001	150ng/ml
tPA	< 0.0001	150ng/ml
PAI1	< 0.0001	150ng/ml

15.0 REFERENCES

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Revision: 0 **Date: 2021-JAN-20** **DCO: N/A**

MP Product Code: *12075-300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1ml	1ml
	C)	12ml	2 (12ml)
	D)	13ml	2 (13ml)
	E)	1 plate	2 plates
	F)	1 (20ml)	1 (20ml)
	G)	1 (7ml)	2 (7ml)
	H)	1 (7ml)	2 (7ml)

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IVD 2° 8° CE
EC REP **CEpartner4U,** Esdoornlaan 13 3951 DBMaarn, The Neatherlands **www.cepartner4u.eu**

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device

2° 8° — Temperature Limitation Storage Condition (2-8° C)

Consult Instructions for Use

REF — Catalogue Number

Σ — Contains Sufficient Test for Σ

LOT — Batch Code

Used By (Expiration Day)

Date of Manufacturer

Manufacturer

EC REP — Authorized Rep in European Country

CE — European Conformity