D-Dimer (human) ELISA Kit

Item No. 502470

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GENERAL INFORMATION

Materials Supplied

Item Number	Item Name	96 wells Quantity/Size	Storage Temperature
400673	D-Dimer (human) ELISA Standard	2 vials	4°C
400674	Anti-D-Dimer (human) ELISA Strip Plate	1 plate	4°C
400675	Anti-D-Dimer (human) Biotin Conjugate	1 vial/0.3 ml	4°C
400664	Streptavidin Poly-HRP	1 vial/1.5 ml	4°C
400108	Immunoassay Buffer D Concentrate (5X)	1 vial/10 ml	4°C
400074	TMB Substrate Solution	1 vial/12 ml	4°C
10011355	HRP Stop Solution	1 vial/12 ml	RT
400035	Polysorbate 20	1 vial/3 ml	RT
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400012	96-Well Cover Sheet	3 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's D-Dimer (human) ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

The stop solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.* safety glasses, gloves, and lab coat) when using this material.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm
2. An orbital microplate shaker
3. Adjustable pipettes; multichannel or repeating pipettor recommended
4. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
5. Materials used for **Sample Preparation** (see page 11)

INTRODUCTION

Background

D-dimer is a fibrin degradation product formed during fibrinolysis.[1,2] It consists of two identical subunits derived from cross-linked fibrin molecules within stable thrombi and is the final fragment from plasmin-mediated degradation of cross-linked fibrin. Plasma levels of D-dimer are commonly used as biomarkers of thrombosis.[2,3] D-dimer is detectable at low levels in plasma in healthy individuals due to the physiological conversion of fibrinogen to fibrin, and these basal levels increase with age.[3] Plasma levels of D-dimer are increased in patients with various types of venous thromboembolism, including pulmonary embolism (PE) and deep vein thrombosis (DVT), but vary dependent on clot burden, timing of measurement, and initiation of interventional treatment. They are also increased in patients with severe COVID-19, a respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), compared to those without severe disease.[4]

About This Assay

Cayman's D-Dimer (human) ELISA Kit is an immunometric (*i.e.* sandwich) assay that can be used for the quantification of D-dimer in human plasma and serum. The standard curve spans the range of 0-400 ng/ml, with a limit of detection of 4.77 ng/ml.

Principle Of This Assay

This immunometric assay is based on a double-antibody "sandwich" technique. Each well of the microwell plate supplied with the kit has been coated with a monoclonal antibody specific for D-dimer. If D-dimer is present in the sample, it will be captured by this immobilized antibody in the first incubation step. A biotin-conjugated D-dimer detection antibody added to the well binds to the D-dimer, forming a 'sandwich'. Excess reagents are washed away and a horseradish peroxidase (HRP)-conjugated streptavidin reagent is added to the well. HRP-conjugated streptavidin binds to the biotin, allowing for quantification of human D-dimer in the sample. The concentration of bound streptavidin is determined by measuring the enzymatic activity of HRP using the chromgenic substrate 3,3',5,5'-tetramethylbenzidine (TMB). After a sufficient period, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of the color is directly proportional to the amount of bound antibody-biotin conjugate, which is proportional to the concentration of D-dimer.

$$\text{Absorbance} \propto [\text{streptavidin-HRP}] \propto [\text{anti-D-dimer biotin}] \propto [\text{D-dimer}]$$

A schematic of this process is shown in Figure 1, on page 8.

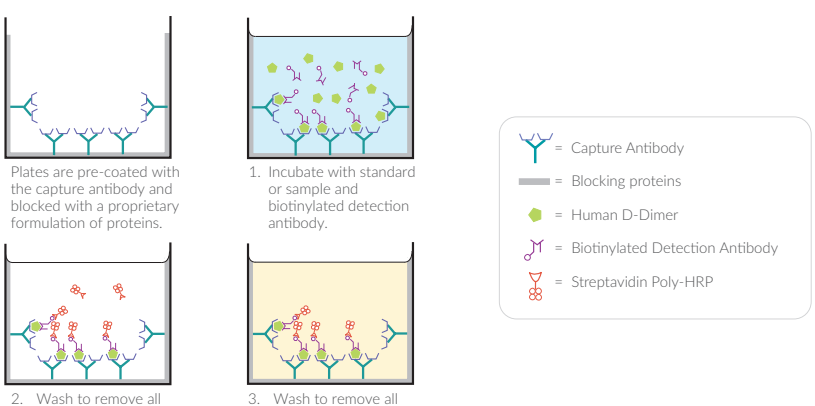


Figure 1. Schematic of the ELISA

Definition of Key Terms

Standard Curve: a plot of the absorbance values versus concentration of a series of wells containing various known amounts of analyte.

Lower Limit of Detection (LLOD): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

Lower Limit of Quantification (LLOQ): the lowest standard concentration in which absorbance (450 nm) – (1.64 x S.D.) is higher than the mean zero value of absorbance (450 nm) + (1.64 x S.D.).

Dilutional Linearity (%):

$$\left[\frac{\text{observed concentration} * \text{dilution factor}}{\text{first observed concentration in the dilution series} * \text{dilution factor}}\right] \times 100$$

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for approximately two months.

1. **Immunoassay Buffer D (1X) Preparation**

   Dilute the contents of one vial of Immunoassay Buffer D Concentrate (5X) (Item No. 400108) with 40 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

2. **Wash Buffer (1X) Preparation**

   Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L and add 1 ml of Polysorbate 20. *NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.*

Sample Preparation

Plasma

Collect blood in vacutainers containing heparin, EDTA, or sodium citrate for plasma samples. To obtain plasma, centrifuge blood at 1,000 x g for 15 minutes at 4°C. Transfer the plasma (upper layer) to a clean tube without disturbing the white buffy layer. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C. Prior to the assay dilute plasma samples with Immunoassay Buffer D (1X) to be in the range of the standard curve.

Serum

Collect blood in vacutainers without a coagulant for serum samples. Allow samples to clot undisturbed for 30-60 minutes at room temperature. To obtain serum, centrifuge at 1,000-2,000 x g for 15-30 minutes 4°C. Transfer the serum (upper layer) to a clean tube, being careful not to disturb the white buffer layer. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C. Prior to the assay dilute plasma samples with Immunoassay Buffer D (1X) to be in the range of the standard curve.

Testing for Interference

This assay has been validated in plasma and serum. Other sample matrices could cause interference and may require dilution. It is recommended to test for interference before embarking on a large number of measurements. To test for interference, dilute one or two test samples with Immunoassay Buffer D (1X) to obtain several different dilutions for each sample. The dilution factor where the change in the final calculated D-dimer concentration is consistent, differing by 20% or less than the previous dilution, is the minimum required dilution for that particular sample type.

Sample Matrix Properties

Parallelism

To assess parallelism, human plasma and serum samples were assayed at multiple dilutions in Immunoassay Buffer D (1X) using the D-Dimer (human) ELISA Kit. Concentrations were plotted as a function of sample dilution. The results are shown below.

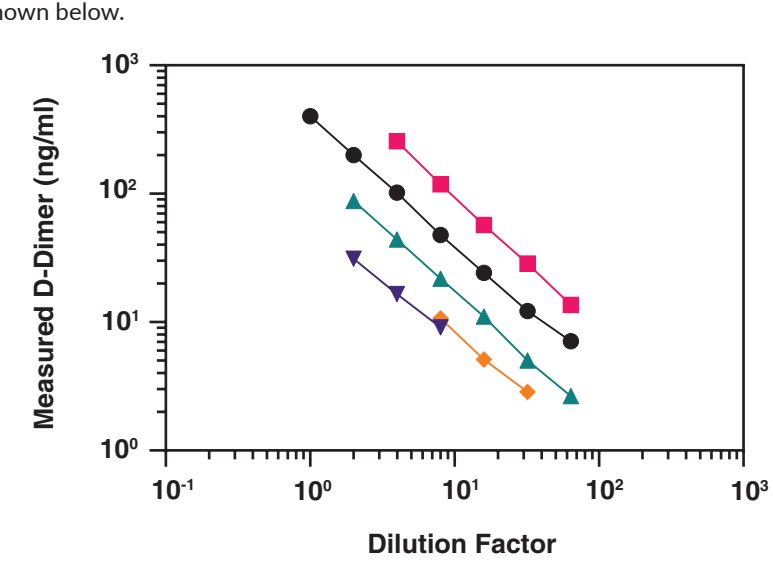


Figure 2. Parallelism of human plasma and serum in the D-Dimer (human) ELISA Kit

Spike and Recovery

Human plasma and serum were spiked with D-dimer, diluted with Immunoassay Buffer D (1X), and analyzed using the D-Dimer (human) ELISA Kit. The error bars represent standard deviations obtained from multiple dilutions of each sample. The results are shown below.

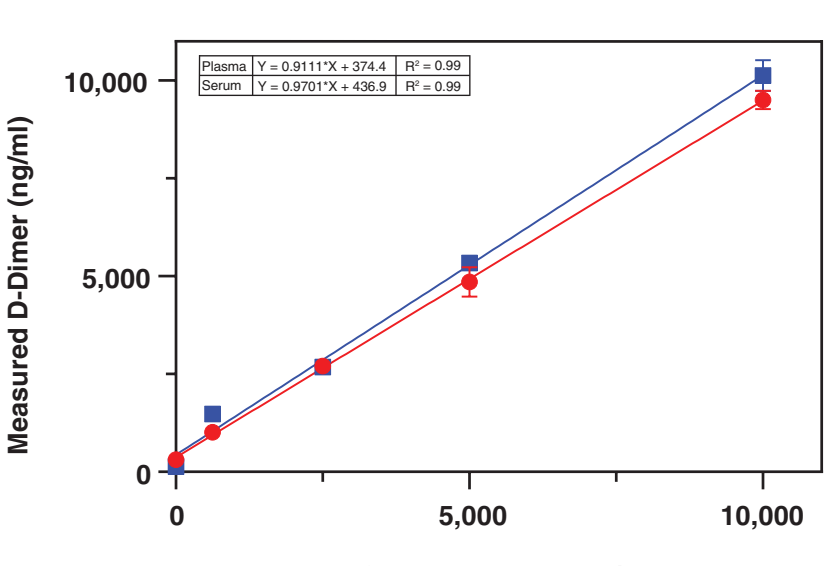


Figure 3. Spike and recovery in human plasma and serum

Dilutional Linearity

Human serum and plasma spiked with D-dimer were analyzed at multiple dilutions using the D-Dimer (human) ELISA Kit. The results are shown in the tables below.

Dilution	Measured Concentration (ng/ml)	Dilutional Linearity (%)
Serum (Spiked with 10,000 ng/ml)		
40	9,525	100
80	9,729	102
160	9,560	100
Serum (Spiked with 5,000 ng/ml)		
20	4,524	100
40	4,777	106
80	5,269	116
Serum (Spiked with 2,500 ng/ml)		
10	2,670	100
20	2,717	102
40	2,724	102

Table 1. Dilutional linearity in serum

Dilution	Measured Concentration (ng/ml)	Dilutional Linearity (%)
Plasma (Spiked with 10,000 ng/ml)		
40	12,854	100
80	11,818	91.9
160	13,201	103
Plasma (Spiked with 5,000 ng/ml)		
20	5,887	100
40	6,024	102
80	6,719	114
Plasma (Spiked with 2,500 ng/ml)		
10	3,187	100
20	3,378	106
40	3,352	105

Table 2. Dilutional linearity in EDTA plasma

Preparation of Assay-Specific Reagents

D-Dimer (human) ELISA Standard

To prepare the standard for use in ELISA: Reconstitute one vial of lyophilized D-Dimer ELISA Standard (Item No. 400673) with 0.4 ml of Immunoassay Buffer D (1X) and mix gently by inversion or pipetting. The concentration of this solution will be 400 ng/ml, which will be S1. Obtain seven clean test tubes and label them #2-8. Aliquot 200 µl Immunoassay Buffer D (1X) to tubes #2-8. Transfer 200 µl of the S1 standard (400 ng/ml) to tube #2 and mix gently. Serially dilute the standard by removing 200 µl from tube #2 and placing it in tube #3; mix gently. Repeat this process for tubes #4-7. Do not add any D-Dimer Standard to tube #8. This tube is the background control. The diluted standards will be stable for 3 hours at room temperature.

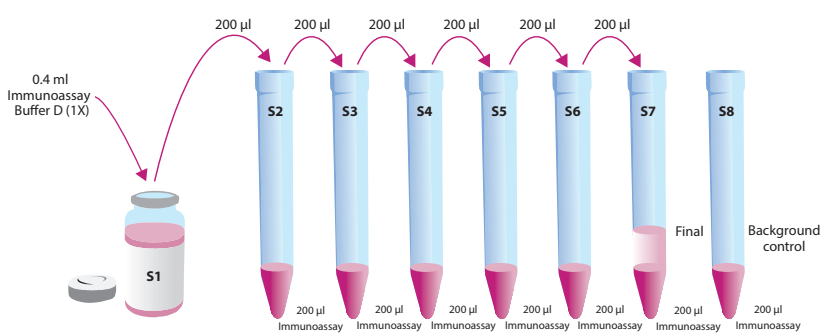


Figure 4. Preparation of D-dimer (human) standards

Anti-D-Dimer (human)-Biotin Conjugate (1X)

Anti-D-Dimer (human)-Biotin Conjugate (Item No.400675) is supplied as a concentrated (20X) stock solution of D-dimer antibody conjugated to biotin. For a full plate, dilute 250 µl of the biotin conjugate with 4.75 ml of Immunoassay Buffer D (1X); for a half plate, dilute 125 µl of the biotin conjugate with 2.375 ml of Immunoassay Buffer D (1X) to make a 1X working solution. Discard any unused biotin conjugate (1X). Store unused Anti-D-Dimer-Biotin Conjugate (20X) stock solution at 4°C.

Streptavidin Poly-HRP (1X)

Streptavidin Poly-HRP (Item No. 400664) is supplied as a concentrated (10X) stock solution of streptavidin conjugated to HRP. For a full plate, dilute 1,200 µl of Streptavidin Poly-HRP (10X) with 10.8 ml of Immunoassay Buffer D (1X); for a half plate, dilute 600 µl of Streptavidin Poly-HRP (10X) with 5.4 ml of Immunoassay Buffer D (1X) to make a 1X working solution. Discard any unused Streptavidin Poly-HRP (1X). Store Streptavidin Poly-HRP (10X) stock solution at 4°C.

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain an eight-point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed in duplicate. For statistical purposes, assaying the samples in triplicate is recommended.

A suggested plate format is shown in Figure 5, below. The user may vary the location and type of wells present as necessary for each particular experiment. It is suggested that the contents of each well be recorded on the template sheet provided (see page 29).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	1	1	1	9	9	9	17	17	17	25
B	S2	S2	2	2	2	10	10	10	18	18	18	25
C	S3	S3	3	3	3	11	11	11	19	19	19	25
D	S4	S4	4	4	4	12	12	12	20	20	20	26
E	S5	S5	5	5	5	13	13	13	21	21	21	26
F	S6	S6	6	6	6	14	14	14	22	22	22	26
G	S7	S7	7	7	7	15	15	15	23	23	23	
H	S8	S8	8	8	8	16	16	16	24	24	24	

S1-S8 = Standard Wells
1-26 = Sample Wells

Figure 5. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Addition of Standards and Samples

Pipette 50 µl of the D-Dimer ELISA Standard or sample into the appropriate wells on the plate. Each sample should be assayed in duplicate, triplicate recommended.

Addition of the Anti-D-Dimer Biotin Conjugate (1X)

Add 50 µl of the Anti-D-Dimer-Biotin Conjugate (1X) working solution to each well of the plate.

Incubation of the plate

Cover the plate with the 96-well Cover Sheet (Item No. 400012) and incubate for one hour at room temperature on an orbital shaker.

Addition of Streptavidin-Poly HRP and second incubation

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X). After the last wash, gently tap the inverted plate on absorbent paper to remove the residual wash buffer (1X).
2. Add 100 µl of the Streptavidin Poly-HRP (1X) working solution to each well of the plate.
3. Cover the plate with a 96-Well Cover Sheet and incubate for 30 minutes at room temperature on an orbital shaker.

Development of the Plate

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X). After the last wash, gently tap the inverted plate on absorbent paper to remove the residual wash buffer.
2. Add 100 µl of TMB Substrate (Item No. 400074) to each well of the plate.
3. Cover the plate with a 96-Well Cover Sheet and incubate for 30 minutes at room temperature on an orbital shaker.
4. DO NOT WASH THE PLATE. Add 100 µl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The stop solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, *etc*.
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Calculations

Plot the Standard Curve and Determine the Sample Concentration

Using computer reduction software, plot absorbance (linear y-axis) versus concentration (linear x-axis) for standards (S1-S8) and graph the data with a quadratic fit. Using the equation of the line, calculate the concentration of D-dimer (human) in each sample, making sure to correct for any sample dilution.

Performance Characteristics

Representative Data

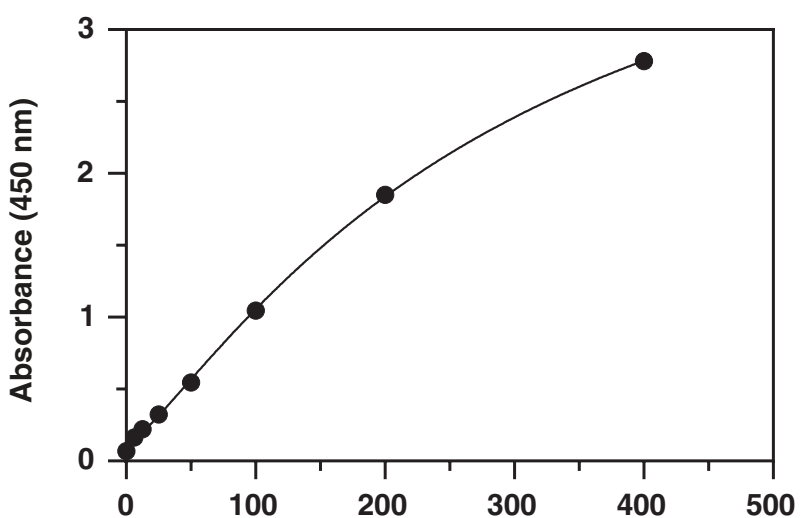
The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples.

Absorbance at 450 nm

D-Dimer Standards (ng/ml)	Absorbance	%CV* Intra-assay Precision	%CV* Inter-assay Precision
400	2.78	6.2	4.1
200	1.85	4.5	1.4
100	1.05	8.8	5.6
50	0.547	9.7	5.6
25	0.324	8.3	3.9
12.5	0.222	11.2	7.3
6.25	0.164	11.4	14.5
0	0.069	--	--

Table 3. Typical results

*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve



Assay Range = 0-400 ng/ml
Sensitivity (defined as LLOQ) = 6.25 ng/ml
Lower Limit of Detection (LLOD) = 4.77 ng/ml

The standards were diluted with Assay Buffer (1X).

Figure 6. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 24 replicates of three matrix controls (human plasma and serum) in a single assay.

Controls	Measured D-Dimer (ng/ml)	%CV
Control 1 (heparin plasma)	11,040	2.9
Control 2 (serum)	482	5.3
Control 3 (citrate plasma)	190	8.4

Table 4. Intra-assay precision

Inter-assay precision was determined by analyzing replicates of three matrix controls (human plasma and serum) in eight separate assays on different days.

Controls	Measured D-Dimer (ng/ml)	%CV
Control 1 (heparin plasma)	12,689	6.9
Control 2 (serum)	491	3.1
Control 3 (citrate plasma)	168	9.1

Table 5. Inter-assay precision

RESOURCES

Troubleshooting

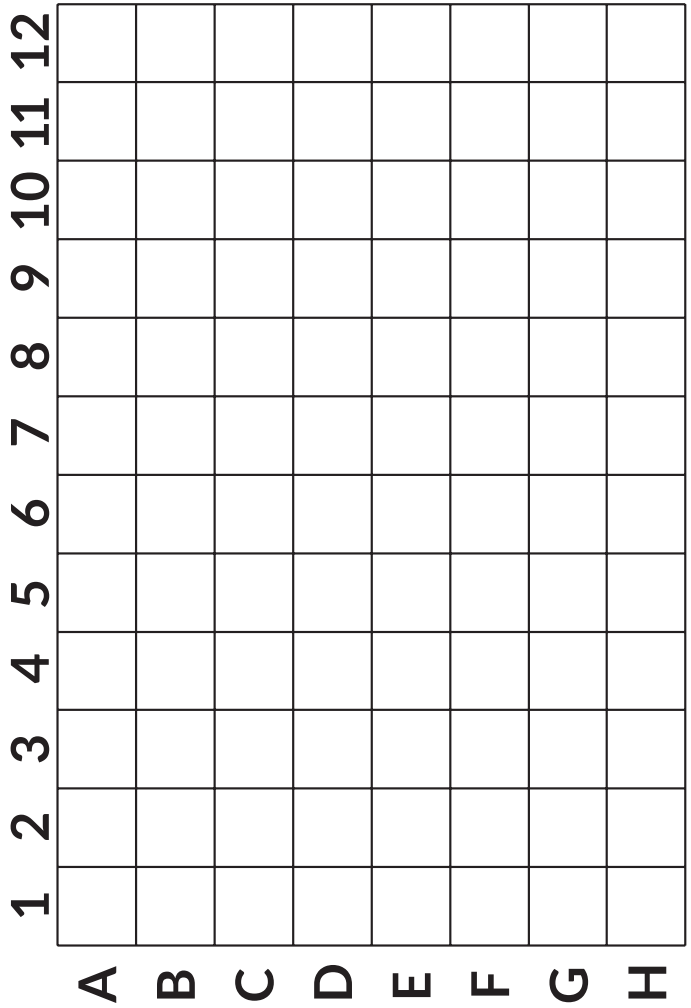
Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
High background wells (>0.150 O.D.)	A. Poor washing B. Exposure of background wells to standards or samples
Poor development (low signal) of standard curve	A. Trace organic contaminants in the water source B. Dilution error in preparing reagents
Poor development (low signal) of samples	Samples are too dilute
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present

References

1. Righini, M., Perrier, A., De Moerloose, P., *et al.* D-dimer for venous thromboembolism diagnosis: 20 years later. J. Thromb. Haemost. **6**, 1059-1071 (2008).
2. Olson, J.D. D-dimer: An overview of hemostasis and fibrinolysis, assays, and clinical applications. *Advances in Clinical Chemistry* **69**, 1-46 (2015).
3. Linkins, L.-A. and Lapner, S.T. Review of D-dimer testing: Good, bad, and ugly. *Int. J. Lab. Hem.* **39(Suppl. 1)**, 98-103 (2017).
4. Lippi, G. and Favaloro, E.J. D-dimer is associated with severity of coronavirus disease 2019: A pooled analysis. *Thrombosis and Haemostasis* **120**, 876-877 (2020).

Procedure	Standards/Samples
Mix all reagents gently	--
Add standards/samples to plate	50 µl
Add Anti-D-Dimer Biotin Conjugate (1X)	50 µl
Incubate	Seal plate and incubate plate for 1 hour at RT on an orbital shaker
Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)
Apply Streptavidin-Poly HRP Solution (1X)	100 µl
Incubate	Seal plate and incubate plate for 30 minutes at RT on an orbital shaker
Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)
Apply TMB substrate	100 µl
Develop	Seal plate and incubate for 30 minutes at RT on an orbital shaker, protected from light
Do not wash, Add HRP Stop Solution	100 µl
Read	Read absorbance at 450 nm

Table 6. Assay Summary



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.