K-*ASSAY*®

D-Dimer

For the Quantitative Determination of Cross-Linked Fibrin Degradation Products Containing D-Dimer

Cat. No. KAI-090

INTENDED USE

The **K-*ASSAY*®** D-Dimer Assay is an *in vitro* diagnostic reagent for the quantitative determination of cross-linked fibrin degradation products containing D-dimer in human plasma or serum by immunoturbidimetric assay. FOR *IN VITRO* DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

During fibrinolysis, plasmin breaks down fibrin and fibrinogen. When insoluble fibrin is degraded, a variety of cross-linked fibrin degradation products (XL-FDP) are produced. The smallest cross-linked fibrin degradation product is D-dimer, a fragment that contains one intermolecular cross-link between the gamma chains of two fibrin monomers. This cross-linkage only occurs in fibrin, but not in fibrinogen, so D-dimer is a specific degradation product of fibrin.[1]

Quantitative D-dimer determination aids in detecting the presence and degree of intravascular coagulation and fibrinolysis (the dissolution of the fibrin in a blood clot) and in monitoring the therapy for disseminated intravascular coagulation (nonlocalized clotting in the blood vessels.) D-dimer is also routinely used for excluding deep venous thrombosis.[2]

PRINCIPLE OF TEST

Latex particles coated with antibody specific to human D-dimer fragment D form immune complexes in the presence of D-dimer from the sample. The immune complexes cause an increase in light scattering, which is proportional to the concentration of D-dimer in the plasma sample. The light scattering is measured by reading turbidity at 570 nm. The sample D-dimer concentration is determined versus dilutions of a D-dimer calibrator of known concentration.

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Buffer Reagent
2-amino-2-hydroxymethyl-1, 3-propadiol , Sodium Azide 0.09%

R2: Latex Suspension
Latex suspension / Anti-human D-Dimer mouse monoclonal antibody, Sodium Azide 0.09%

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE. Rx only.

Not to be used internally in humans or animals. Normal precautions exercised in handling laboratory reagents should be followed. Do not mix or use reagents from one test kit with those from a different lot number. Do not use reagents past their expiration date stated on each reagent container label.

Do not pipette by mouth. Avoid ingestion and contact with skin.

Reagents in this kit contain sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water.

REAGENT PREPARATION

Reagents are ready to use and do not require reconstitution. Mix before using by gently inverting the bottles. After opening, gently invert Reagent 2 once a week.

STORAGE AND STABILITY

All reagents should be stored at 2-8℃ and protected from light. Unopened reagents can be used for one year from the date of manufacture as indicated on the expiration date on the package and bottle labels if stored at 2-8℃. Once the reagent vial has been opened, store tightly capped at 2-8℃ and use within 1 month. Discard reagents if they become contaminated. Evidence of obvious precipitation in reagent 2 (R-2) solution is cause to discard.

SPECIMEN COLLECTION AND PREPARATION

Plasma
Whole blood is collected in sodium citrate anticoagulant. After collection, immediately centrifuge. In the U.S., follow NCCLS guideline H3-A2. Plasma samples can be stored for 1 week at 4℃.

Serum
Blood should be collected from a patient and the serum separated as soon as possible. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the serum separated from the clot to a plastic tube (not glass). It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. Samples can be stored for 1 week at 4℃.

Use plastic tubes for storing the samples. Do not use glass.

AUTOMATED ANALYZER APPLICATION

Suitable for two-reagent automated analyzers that can measure a rate reaction at an absorbance of 570 nm. Refer to the instrument manual from the manufacturer regarding the following:

a) Use or function
b) Installation procedures and requirements
c) Principles of operation
d) Performance characteristics, operating instructions
e) Calibration procedures including materials and / or equipment to be used
f) Operational precautions, limitations, and hazards
g) Service and maintenance information

PROCEDURE

Materials Supplied

KAI-090
Reagent 1 (R-1) Buffer Reagent 1 x 16 mL
2-amino-2-hydroxymethyl-1,3-propadiol
Reagent 2 (R-2) Latex Suspension 1 x 8.5 mL
Latex suspension / Anti-D-Dimer mouse monoclonal antibody

Materials Required But Not Supplied

Calibrators: **K-*ASSAY*®** D-Dimer Calibrator, Cat. No. KAI-091C.

Purified water.

Two Reagent Clinical Chemistry Analyzer:
Capable of accurate absorbance readings at 570 nm
Capable of accurately dispensing the required volumes
Capable of maintaining 37℃

Plastic tubes.

Saline for use in sample dilution, 150 mM Sodium Chloride
pH 7.0 – 7.2.

Assay Procedure

An example of standard protocol automated application:

Sample 3.5 µL
↓
• ← R1 (Buffer Reagent) 140 µL
↓ 37℃, 279 seconds
• ← R2 (Latex Suspension) 70 µL
↓ 37℃, 304 seconds
Start read: average (304.8 and 322.6 seconds), 570 nm
Final read: average (569.5 and 587.4 seconds), 570 nm

Note: Allow all reagents and specimens to warm to room temperature (18-25℃). Mix all reagents gently before using.

Automated Method

Parameters for automated analyzers are available.

CALIBRATION

A multi-point calibration curve should be made using the **K-*ASSAY*®** D-Dimer Calibrator. It is recommended that the user determine calibration curve frequency as this depends on the instrument and type/number of other assays being performed. Initially, calibration should be performed each day.

QUALITY CONTROL

It is recommended that at least two levels of control (with known concentrations of D-Dimer) be included in all assay runs.

CALCULATIONS

D-Dimer levels are determined by the analyzer using the prepared calibration curve.

LIMITATIONS OF PROCEDURE

If D-Dimer value of sample is greater than the highest calibrator value, dilute with saline and re-assay. Expected values listed apply only to serum samples tested in an Asian population. In addition to cross-linked fibrin degradation products containing D-Dimer, this assay also may react with fragments X and Y.

PERFORMANCE

Sensitivity

When a saline blank is used as a sample, the absorbance is below 0.003 / min. When a calibrator having a D-Dimer concentration of around 10 µg/mL is assayed, the absorbance (after subtracting the saline blank) is within the range of 0.005 to 0.045 / min.

Specificity

When a sample with a known value is assayed, the result is within ±10% of the assigned value.

Precision Assay

(Within Run)
The following results were obtained on a Hitachi 717 analyzer with pooled human plasma:

	Serum		
	Sample I	**Sample II**	**Sample III**
N	5	5	5
Mean	2.29 µg/mL	9.60 µg/mL	22.04 µg/mL
Std. Dev.	0.06	0.14	0.36
CV	2.7%	1.4%	1.6%

	Plasma		
	Sample A	**Sample B**	**Sample C**
N	10	10	10
Mean	3.55 µg/mL	11.34 µg/mL	21.12 µg/mL
Std. Dev.	0.09	0.12	0.12
CV	2.5%	1.1%	0.6%

(Between Runs)
The following results were obtained on a Hitachi 717 analyzer with pooled human plasma:

	Serum		
	Sample I	**Sample II**	**Sample III**
N	10	10	10
Mean	2.44 µg/mL	10.19 µg/mL	28.43 µg/mL
Std. Dev.	0.06	0.19	0.27
CV	2.5%	1.9%	0.95%

	Plasma		
	Sample A	**Sample B**	**Sample C**
N	5	5	5
Mean	3.60 µg/mL	11.56 µg/mL	21.55 µg/mL
Std. Dev.	0.08	0.11	0.28
CV	2.27%	0.96%	1.30%

Accuracy / Correlation

A comparison of the ***K-ASSAY®*** D-Dimer reagent (KAI-090) and the ***K-ASSAY®*** D-Dimer reagent (KAI-078) was performed with the following results:

y = 0.964x – 0.1742
r = 0.9801
n = 80
x = ***K-ASSAY®*** D-Dimer (KAI-078)
y = ***K-ASSAY®*** D-Dimer (KAI-090)

Lower Limit of Detection
The lower limit of detection is 0.5 µg/mL DDU.

Assay Range
0.5 µg/mL to 30 µg/mL DDU (or value of highest calibration point)

INTERFERENCE

Bilirubin F	No interference up to 18 mg/dL
Bilirubin C	No interference up to 21.2 mg/dL
Hemoglobin	No interference up to 500 mg/dL
RF	No interference up to 520 IU/mL
Citric Acid (sodium)	No interference up to 500 mg/dL
Ascorbic Acid	No interference up to 100 mg/dL
NaF	No interference up to 400 mg/dL
Heparin	No interference up to 400 mg/dL
EDTA-2Na	No interference up to 100 mg/dL
Chyle (Formazine Turbidity)	No interference up to 3,000
Fibrinogen	No interference up to 500 mg/dL

EXPECTED VALUES

In our laboratory, the expected value of D-Dimer in normal plasma is less than 0.463 µg/mL DDU for 88 normal plasma samples from patients ages 33-58. It is recommended that each laboratory establish its own expected range to reflect its patient population.

REFERENCES

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LABELING SYMBOLS

LOT	Lot Number
RGT	Reagent
	Expiration or "Use By" Date
REF	Catalog Number
IVD	For *In Vitro* Diagnostic Use
2-8°C	Temperature Limitation. Store between 2 and 8 degrees C
	Manufacturer
	Consult Package Insert for Instructions for Use
EC REP	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

CE

EC REP

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