**K-*ASSAY*®**

# High-Sensitive D-Dimer

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

**Cat. No. KAI-102**

### INTENDED USE

The **K-*ASSAY*®** High-Sensitive D-Dimer Assay is for the quantitative determination of cross-linked fibrin degradation products containing D-dimer in human serum or citrated plasma by immunoturbidimetric assay.
NOT FOR USE IN THE U.S.

### INTRODUCTION AND SUMMARY

During fibrinolysis, plasmin breaks down fibrin and fibrinogen. When insoluble fibrin is degraded, a variety of cross-linked fibrin degradation products are produced. The smallest cross-linked fibrin degradation product is D-dimer (DD), a fragment that contains one intermolecular cross-link between the gamma chains of two fibrin monomers. Another cross-linked fibrin degradation product is DD•E, consisting of fragment E non-covalently associated with D-Dimer. 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 a monoclonal antibody specific to human D-dimer and DD•E 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 sample. The light scattering is measured by reading turbidity at 500 to 600 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
Tris-HCl buffer, sodium azide 0.05%

R2: Antibody Reagent
latex suspension / anti-human D-Dimer mouse monoclonal antibody, sodium azide 0.05%

### WARNINGS AND PRECAUTIONS

NOT FOR USE IN THE U.S.

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. The buffer solution is weakly alkaline (pH = 8.3). Avoid direct contact to skin and eyes. If contact occurs, flush with copious amounts of water and seek medical attention if necessary.

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.

For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control, Atlanta, Georgia.

### REAGENT PREPARATION

Reagents are ready to use and do not require reconstitution. Before use, gently invert Reagent 2 at least once a week.

### STORAGE AND HANDLING

All reagents should be stored at 2-8°C. Return all reagents to 2-8°C promptly after use.

### REAGENT STABILITY

Unopened reagents can be used for 18 months from the date of manufacture as indicated on the expiration date on the package and bottle labels if stored at 2-8°C. Once the reagent vial has been opened, store tightly capped at 2-8°C 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 a tube containing 3.2% buffered sodium citrate (blue-top). After collection, immediately mix the sample with the anticoagulant by gently inverting the tube at least six times. Centrifuge and carefully remove the plasma. In the U.S., follow NCCLS guideline H3-A2. Plasma samples should be assayed within 24 hours, or stored frozen until they can be tested.

**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. Blood should be centrifuged immediately after drawing and tested on the same day. If testing cannot be completed within 24 hours, the sample should be stored frozen.

### AUTOMATED ANALYZER APPLICATION

Suitable for two-reagent automated analyzers that can measure a rate reaction at an absorbance of 500 to 600 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-102**

| | |
|---|---|
| Reagent 1 (R-1) Buffer Reagent | 2 x 18 mL |
| Reagent 2 (R-2) Antibody Reagent | 1 x 7 mL |

**Materials Required But Not Supplied**

Calibrators: **K-*ASSAY*®** High-Sensitive D-Dimer Calibrator, Cat. No. KAI-103C

Two Reagent Clinical Chemistry Analyzer:
Capable of accurate absorbance readings at 500-600 nm
Capable of accurately dispensing the required volumes
Capable of maintaining 37°C

Pipettes: capable of accurately dispensing the required volumes

Test Tubes: glass or plastic

**Assay Procedure**

An example of standard protocol automated application:

| | |
|---|---|
| Sample | 20 µL |
| ↓ | |
| • ← R1 (Buffer Reagent) | 144 µL |
| ↓ 37°C, 5 min | |
| • ← R2 (Antibody Reagent) | 40 µL |
| ↓ 37°C, 5 min | |

2-point endpoint, 546nm

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

**Automated Method (Example)**

Chemistry Parameters for Automatic Analyzer (Hitachi 902):

| | |
|---|---|
| 1. TEST: [ HSDD ] | 35. REAGENT BLANK ABS. LIMIT: [ 32000 ] |
| 2. ASSAY CODE: [ 2 POINT END ] | |
| 3. TEST: [ 0 ] | 36. CALCULATION FACTOR K: [ + ] |
| 4. REACTION TIME: [ 10 ] | 37. CALCULATION FACTOR K2: [ 0 ] |
| 5. ASSAY POINT 1: [ 20 ] | 38. CALCULATION FACTOR K3: [ 0 ] |
| 6. ASSAY POINT 2: [ 24 ] | 39. CALCULATION FACTOR K4: [ 0 ] |
| 7. ASSAY POINT 3: [ 20 ] | 40. CALCULATION FACTOR K5: [ 0 ] |
| 8. ASSAY POINT 4: [ 21 ] | 41. CALCULATION FACTOR A: [ 0 ] |
| 9. SUB WAVELENGTH: [ 0 ] | 42. CALCULATION FACTOR B: [ 0 ] |
| 10. MAIN WAVELENGTH: [ 546 ] | 43. CALCULATION FACTOR C: [ 0 ] |
| 11. SAMPLE VOLUME: [ 20.0 ] | 44. SD. LIMIT: [ 999 ] |
| 12. REAGENT VOLUME (R1): [ 144 ] | 45. DUPLICATE LIMIT: [ 32000 ] |
| 13. REAGENT POSITION (R1): [ # ] | 46. SENSITIVITY LIMIT: [ 0 ] |
| 14. REAGENT BOTTLE SIZE (R1): [ S or L ] | 47. ABS. LIMIT (LOWER): [ -32000 ] |
| 15. REAGENT VOLUME (R2): [ 0 ] | 48. ABS. LIMIT (UPPER): [ 32000 ] |
| 16. REAGENT POSITION (R2): [ 0 ] | 49. REACTION LIMIT: [ 32000 ] |
| 17. REAGENT BOTTLE SIZE (R2): [ S or L ] | 50. REACTION LIMIT: [ INCREASE ] |
| 18. REAGENT VOLUME (R3): [ 40 ] | 51. PROZONE LIMIT: [ 57 ] |
| 19. REAGENT POSITION (R3): [ # ] | 52. PROZONE LIMIT: [ LOWER ] |
| 20. REAGENT BOTTLE SIZE (R3): [ S or L ] | 53. PROZONE FINAL ENDPOINT: [ 29 ] |
| 21. CALIB. TYPE: [ SPLINE ] | 54. STD. VALUE RANGE UPPER LIMIT: [ -99999 ] |
| 22. INSTRUMENT FACTOR: [ 0 ] | |
| 23. STD. CONCENTRATION 1: [ 0.0 ] | 55. STD. VALUE RANGE LOWER LIMIT: [ 999999 ] |
| 24. STD. POSITION 1: [ # ] | |
| 25. STD. CONCENTRATION 2: [ *2 ] | 56. INSTRUMENT CONSTANT (a): [ 1.0 ] |
| 26. STD. POSITION 2: [ # ] | 58. KEY SETTING: [ ++ ] |
| 27. STD. CONCENTRATION 3: [ *3 ] | |
| 28. STD. POSITION 3: [ # ] | |
| 29. STD. CONCENTRATION 4: [ *4 ] | |
| 30. STD. POSITION 4: [ # ] | |
| 31. STD. CONCENTRATION 5: [ *5 ] | |
| 32. STD. POSITION 5: [ # ] | |
| 33. STD. CONCENTRATION 6: [ *6 ] | |
| 34. STD. POSITION 6: [ # ] | |

# = User Defined
*2-6: Input concentration of calibrators
+: If a new calculation is being done, input a value of 10000. Analyzer will automatically calculate value for future use.
++: Input value for key setting (1-38)

Parameters for other automated analyzers are available.

### CALIBRATION

A multi-point calibration curve should be made using the **K-*ASSAY*®** High-Sensitive 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**

A quality control program is recommended for all clinical testing laboratories. It is recommended that at least two levels of control (with known concentrations of D-Dimer) be included in all assay runs.

Two levels of quality control material of known values should be run according to state, federal, and accreditation requirements or whenever there are questionable results or instrument performance, after analyzer maintenance or manufacturer's service, with each new lot of reagent, and at a minimum of every 30 days for opened vials to check storage conditions.

The values obtained for controls should ideally fall within the manufacturer's specified range. However, due to differences in assays and analyzers used to assay a control by the control manufacturer, a laboratory may establish its own control ranges by assaying the controls a sufficient number of times to generate a valid mean and acceptable range.

**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.

This assay has not been evaluated for its ability to aid in the diagnosis of venous thromboembolism diease, or for its ability to rule out venous thromboembolism disease.

**PERFORMANCE**

**Precision Assay**

The within-run, between-run, and total precision for the ***K-ASSAY*** ® High-Sensitive D-Dimer assay was determined using packaged reagents, human serum samples and controls, and a Roche / Hitachi 902 analyzer in accordance with CLSI EP5-A2.

| | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| N | 80 | 80 | 80 |
| Mean (ng/mL) | 178.7 | 923.4 | 2,632.0 |
| Within Run S.D. | 10.7 | 14.4 | 30.7 |
| Within Run C.V. | 6.0 % | 1.6 % | 1.2 % |
| Between Run S.D. | 10.2 | 18.6 | 39.5 |
| Between Run C.V. | 5.7 % | 2.0 % | 1.5 % |
| Total S.D. | 10.4 | 30.0 | 45.9 |
| Total C.V. | 5.8 % | 3.2 % | 1.7 % |

| | Sample 4 | Sample 5 | Sample 6 |
|---|---|---|---|
| N | 80 | 80 | 80 |
| Mean (ng/mL) | 5,456.3 | 6,616.4 | 7,346.2 |
| Within Run S.D. | 113.6 | 176.8 | 146.6 |
| Within Run C.V. | 2.1 % | 2.7 % | 2.0 % |
| Between Run S.D. | 92.1 | 77.2 | 154.8 |
| Between Run C.V. | 1.7 % | 1.2 % | 2.1 % |
| Total S.D. | 173.2 | 245.0 | 249.1 |
| Total C.V. | 3.2 % | 3.7 % | 3.4 % |



**Accuracy / Correlation**

Testing was performed on a Roche / Hitachi 902 analyzer using unaltered, human citrated plasma samples and in accordance with the CLSI EP9-A2 guideline. A comparison of the ***K-ASSAY*** ® High-Sensitive D-Dimer and Company X's D-Dimer assay performed with the following results.

Linear Regression:
y = 0.6309x – 130.5981
r = 0.9927
n = 44
x = Company X's D-Dimer
y = ***K-ASSAY*** ® High-Sensitive D-Dimer

**Linearity**

Testing was performed on a Roche / Hitachi 917 analyzer according to the CLSI EP6-A guideline. A high D-Dimer human serum pool was serially diluted with diluent to make 14 samples between 30 – 8,100 ng/mL and each sample run 3 times with the following results.

First order regression:
y = 1.0081x + 23.608
r = 0.9996
Standard Error of Regression = 79.794

**Assay Range**

Testing was performed on a Roche / Hitachi 902 analyzer according to the CLSI EP17-A guideline using native and diluted human pooled serum samples with the following results.

Limit of Blank (LoB) = 5.1 ng/mL
Limit of Detection (LoD) = 12.9 ng/mL
Limit of Quantitation (LoQ) = 30.0 ng/mL

Assay Range: 30 – 8,000 ng/mL DDU
0.030 - 8.000 µg/mL DDU
(using LoQ as lower limit and highest calibrator as upper limit)

**INTERFERENCE**

Testing was performed on a Roche / Hitachi 902 analyzer in accordance with the CLSI EP7-A2 guideline with the following results.

Criteria : Recovery within ± 10% of initial value

| | |
|---|---|
| Ascorbic Acid: | No interference ≤ 30 mg/dL |
| Bilirubin C: | No interference ≤ 200 mg/dL |
| Bilirubin F: | No interference ≤ 180 mg/dL |
| Chyle: | No interference ≤ 1500 FTU |
| Citric Acid: | No interference ≤ 3.8 % |
| EDTA-2A: | No interference ≤ 0.15 % |
| Fibrinogen: | No interference ≤ 1,000 mg/dL |
| Hemoglobin: | No interference ≤ 500 mg/dL |
| Heparin: | No interference ≤ 24 IU/dL |
| Rheumatoid Factor: | No interference ≤ 500 IU/mL |
| Sodium Fluoride: | No interference ≤ 10 mg/dL |

**EXPECTED VALUES**

Based on an in-house study of 121 normal, 3.8% citrated plasma samples (62 male and 59 female), the 95% reference interval for combined male and female is 49 to 794 ng/mL DDU (0.049 - 0.794 µg/mL DDU) using the nonparametric method. There was no significant difference between male and female samples.

Due to population differences, each laboratory should establish its own expected values using this kit.

**REFERENCES**

1. Sandkamp, M. *et al*., *Clin. Chem.* 36:20-23 (1990).
2. Wo, J.H. *et al*., *Clin. Chem.* 39:209-212 (1993).

**LABELING SYMBOLS**

| | |
|---|---|
| LOT | Lot Number |
| RGT | Reagent |
| | Expiration or "Use By" Date |
| REF | Catalog Number |
| 2-8°C | Temperature Limitation. Store between 2 and 8 degrees C |
| | Potential Human Biohazard |
| | 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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